Vol. 18 No. 4

PLANT PHYSIOLOGY

OCTOBER, 1943

A NEW TYPE OF INTERMITTENTLY-IRRIGATED SAND CULTURE EQUIPMENT[1]

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(WITH TWO FIGURES)

A type of sand culture apparatus with automatic flushing suitable for the growth of small plants in the greenhouse has recently been described by EATON.[2] This apparatus fulfills certain conditions necessary in the culture of plants on saline nutrient substrates. By means of frequent irrigations

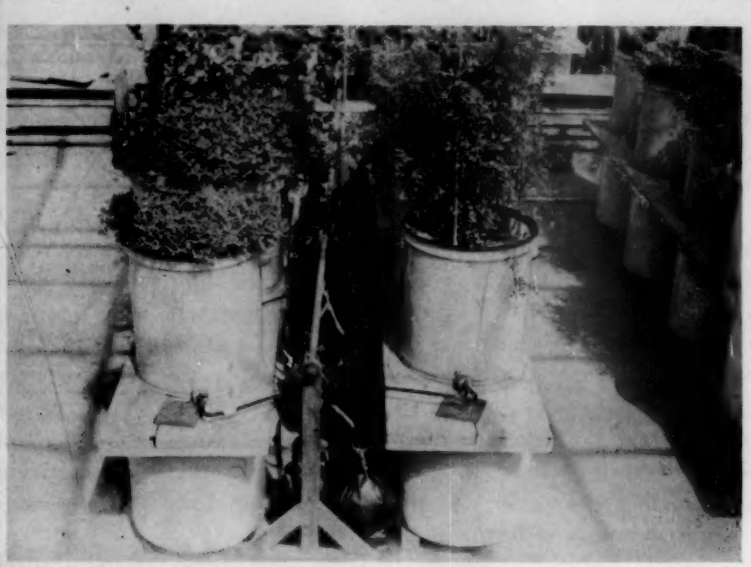

FIG. 1. General view of apparatus with plants.

with an excess of solution, the salt content of the solution in the root zone does not become unduly concentrated as a result of continued water absorption and of evaporation from the surface of the sand.

The present discussion deals with a similar type of greenhouse sand culture equipment of larger dimensions—five times the volume of sand and

[1] Contribution from the U. S. Regional Salinity Laboratory, Bureau of Plant Industry, Soils and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture, Riverside, California.

[2] EATON, FRANK M. Plant culture equipment. Plant Physiol. 16: 385–392. 1941.

twice the volume of nutrient solution—and possessing the advantage that most of the necessary equipment is readily available or easily obtainable under normal conditions. This apparatus has proved successful for growing strawberry and Ladino clovers, alfalfa (fig. 1), beans, flax, and guayule. The size of the sand crock and solution reservoir is amply sufficient, however, for the growth of a mature tomato plant. Very little manual attention is required, and the apparatus has proved relatively trouble-free in operation.

The method of irrigation in this apparatus is very similar to that of various large-scale beds used for commercial or research purposes. The difficulty with such beds, however, is that for research purposes the bed is the statistical unit. Replication is limited, and therefore there is not the degree of statistical precision obtainable with the individual culture units herein described.

Description and operation of apparatus

Each unit is composed of the following main items: A 5-gallon glazed crock (34)[3] filled with sand (35)[4] in which the plant or plants are grown and a 5-gallon glazed crock (21) to serve as the solution reservoir; a half-gallon glass jug (14) which delivers two quarts of nutrient solution to the plants at each irrigation; a check valve (24) to control the direction of flow of solutions; and a compressed air-line (5).

At hourly, or other selected intervals, compressed air is delivered to the glass jug forcing the solution up through the effluent tube (19, 33) to the top of the sand culture and out through the holes in the aluminum ring (29). This pressure on the solution in the jug and in the refill tube (13) forces the glass bead (25) to the bottom of the valve (24) where it forms a seal against the rubber tubing (26) and prevents the escape of solution back into the reservoir (21) during the irrigation. After a lapse of time sufficient for emptying the half-gallon bottle (approximately 2 minutes), the solenoid valve in the air supply line closes, and simultaneously another solenoid valve on the end of a "T" in the system air-line opens, thus relieving the pressure in the air-line (5). At this time solution flows by force of gravity from the reservoir to refill the jug.

The excess of solution delivered to the sand culture returns through a drain tube (18) to the reservoir (21).

Each unit has 26 liters of solution of which approximately 6 liters are retained by the sand, 2 liters in the jug, and approximately 18 liters in the reservoir. By noting the original level of solution in the reservoir, the volume of solution is maintained by the addition of distilled water.

[3] Numbers in parentheses refer to number-labels in figure 2.

[4] The sand should be of a degree of fineness which will prevent too rapid a rate of percolation of water through the sand, since it is desirable to have a sheet of water developed over the surface of the sand at each irrigation. Sand of the following composition has given satisfactory results in our work: not less than 85 per cent. by weight to pass a 20-mesh screen and be retained on a 100-mesh screen.

Description of parts shown in figure 2

A. Glass bead check valve.

1. Rubber tubing sealed to the glass tube (2) with Automotive Bond Cement. The lower end of the rubber tubing is serrated to prevent formation of a seal when the glass bead rises to the top of the valve during refilling of the jug.
2. 10-mm. O.D. glass tubing, 3½ inches in length.
3. Glass bead, 4 mm. in diameter.
4. Rubber tubing sealed to the glass tubing (2) with Automotive Bond Cement. By means of a hot, metal ball bearing, a seat for the glass bead is seared into the upper end of this piece of rubber tubing. After cooling, the excess melted rubber may be removed by use of benzine or other suitable solvent, and then the surface should be thoroughly dusted with talc.

B. Sand culture apparatus.

5. ⅜″ galvanized iron pipe which serves as the air-supply line for each group of 10 cultures (see also fig. 1).
6. ⅛″ copper tubing outlet from main air-line pipe.
7, 9, 11, 22, 27, 32, 36. Rubber tubing connections.
8. Two-inch piece of ½-mm. capillary tubing, selected for uniformity, to serve as a modulator of the air-supply, and thereby ensure uniform air-pressure to all cultures.
10. 3-mm. O.D. glass tubing.
12. Wooden plank, 1″ × 14″ × 8½′, with 2½″ holes approximately 20 inches apart.
13, 18, 19, 33. 5-mm. O.D. glass tubing.
14. One-half gallon glass jug.
15. No. 6 long-taper rubber stopper. (This stopper should be anchored in place by a light wire brace extending over stopper and threaded through the handle of the jug.)
16, 39. No. 3 rubber stopper.
17. Wooden skid, 2″ × 2″ × 8½′.
20. Culture solution.
21, 34. 5-gallon glazed crock.
23, 24, 25, 26. "A–1, –2, –3, –4," respectively.
29. Soft aluminum tubing (3/16″) to form a complete ring with the exception of the arms of the glass T-tube (31) and the rubber tubing connections.
30. 1/32″ outlet hole in the tube. These holes should be spaced about 1½″ apart on the inner surface of the ring (*i.e.*, toward center of crock) and slightly below center so that the solution will be directed downward at about a 45° angle.
31. Glass T-tube.
35. Fine quartz sand (see footnote no. 4).

37. Quartz rock.
38. Wire screen or gauze, 2″ × 2″, with a notch or a hole to accommodate the upright solution-delivery tube (33).

Substitutions: For all "rubber tubing connections" (*i.e.*, 7, 9, 11, 22, 27, 32, 36) windshield wiper hose (cloth inserted rubber) may be substituted. Additional features of this type of hose: Heat resistant; oil resistant; reinforced side walls; kink-proof; and long life.

Saran tubing may be substituted for the soft, aluminum tubing (29).

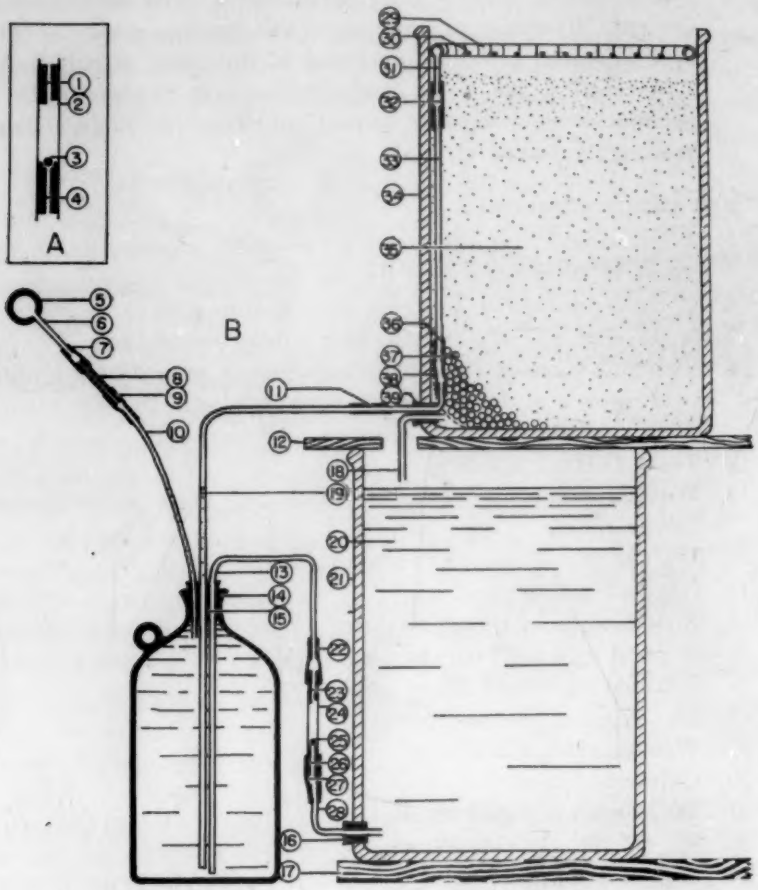

FIG. 2. Cross-section view of sand culture apparatus. (Description in text.)

Source and control of the compressed air

For operating 100 cultures by this method, one should have a source of compressed air which delivers 6 cu. ft. of air per minute at 5 to 7 lb. pressure per square inch. If a source of compressed air is already available but the pressure is in excess of 5 to 7 lb. per square inch, a pressure-control diaphragm valve which will reduce the pressure to the desired level is necessary.

Time switches regulate the time at which this air-delivery valve opens and the length of time it remains open. The following time switches are suitable:

1. AN INTERVAL TIME SWITCH.—An interval time switch can be used alone without a master switch (2, below) if the cultures are to be irrigated each hour throughout a 24-hour period. This type of timer makes a complete cycle every 60 minutes. The duration of the "on" period can be set for any fraction of the 60-minute period. In this switch two circuits are involved—the one being "on" while the other is simultaneously "off," and vice versa. Thus if compressed air is permitted to flow into the line for three minutes during the "on" period of one circuit, then subsequently the solenoid valve which reduces the pressure to atmospheric pressure will be activated and held open for the remainder of the hour (57 minutes) by the other circuit.

2. MASTER SWITCH.—This switch is connected with the interval time switch (1, above) in such a way that the circuit to the solenoid valve controlling the air supply is completed only during the "on" period of both time switches. The "on" and "off" pins on the 24-hour dial of the master switch cannot be inserted nearer together than every 15 minutes. This switch is thus used only to limit the irrigations to selected hours, and the interval time switch determines the length of time during which there will be compressed air in the air-line (5).

If there is no present available source of compressed air, a small diaphragm compressor may be used; then the time switches control the motor on the compressor instead of the solenoid valve on a main air-supply line.

Solution change or solution renewal

Certain small diaphragm compressors, such as are used for paint-spraying outfits, may be used to produce a vacuum instead of a pressure by fixing a hose connection on the air-intake. This vacuum may be conducted to a 5-gallon carboy and a second hose from the carboy used to remove the 18 liters of solution in the reservoir.

Control of algal growth

All external glass tubing (except the valve) should be painted black. It is preferable to wrap the valve with black friction tape so that one may easily check on the functioning of the valve. A heavy paper bag, sprayed inside and out with black paint, should be tied around the glass jug (see fig. 1).

After the seedlings have attained sufficient size, a one-half to one-inch layer of quartz rock may be added over the surface of the sand. These larger particles dry thoroughly between irrigations and the growth of algae on the surface is thus prevented or greatly inhibited.

In order to inhibit the growth of algae in the reservoir, a covering is laid over the hole in the plank through which the drain tube (18) passes (see fig. 1).

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A BIOCHEMICAL STUDY OF CURING PROCESSES IN SWEET POTATOES[1]

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Introduction

If sweet potatoes are to be stored for any length of time they must be subjected immediately after digging to a suitable combination of temperature and humidity for effective curing of the roots. The physiology of curing is imperfectly understood because of our limited information of the essential chemical changes in the roots during curing. Previous studies have been confined chiefly to carbohydrate transformations and respiration. The rate of respiration decreases over that of freshly harvested potatoes (**1, 5**). Both sucrose and reducing sugars accumulate at the expense of the starch (**1, 3, 4**). The percentage of moisture changes very little regardless of the amount of shrinkage by loss of water (**3, 5**). The pH data for expressed juice do not give any significant information on the curing process (**5**).

The present investigation has been concerned with the nitrogen metabolism and pectic transformations in Maryland Golden Sweet Potatoes during curing under different combinations of temperature and humidity and also during subsequent storage under favorable conditions. The keeping qualities of the sweet potatoes cured under the different conditions were recorded in order to determine the relationship of the nitrogen metabolism and pectic changes in the roots during curing to their keeping qualities in storage. The results are also of general physiological interest in relation to the effect of temperature and humidity on nitrogen distribution in fleshy roots.

The sweet potatoes for the experimental lots were carefully gathered in the field and placed under experimental conditions the day following their harvest. The lots were cured for 10 or 11 days under the different combinations of temperature and humidity indicated in the tables. All of the lots at the end of the curing period were stored for four months in an electric refrigerator especially equipped to give the desired temperature control. All the lots were stored at 50° to 53° F. and 50 to 60 per cent. relative humidity. Analyses were made at intervals during the curing and storage periods.

Analytical methods

SAMPLING AND DETERMINATION OF MOISTURE.—Six representative sweet potatoes were ground in a Nixtamal mill, and after thorough mixing, samples of the fresh pulp were weighed out for moisture, pectins, and nitrog-

[1] Scientific Article no. A51, Contribution no. 1881 of the Maryland Agricultural Experiment Station (Department of Botany).

enous constituents. To determine moisture, about 5 gm. of pulp were dried to constant weight at 80° C. in a vacuum of 30 inches of mercury.

TOTAL PECTIN.—Duplicate samples of 50 gm. of the freshly ground pulp were weighed into counterpoised Erlenmeyer flasks and covered with 260 ml. of hot 95 per cent. alcohol. After the storage alcohol had been removed by filtration the samples were washed with alcohol and ether, dried at 60° C. in a vacuum oven, and ground to pass a 60-mesh sieve. A 2-gm. sample of the dried material was placed in a 500-ml. Erlenmeyer flask, covered with 100 ml. of 0.5 per cent. ammonium citrate solution, and boiled very gently for 30 minutes. The solutions were filtered into a 500-ml. volumetric flask. The pulp was washed with warm water, returned to the Erlenmeyer flask with 100 ml. of N/30 hydrochloric acid, and refluxed gently for one hour. The material was again filtered and washed with warm water. The filtrates were combined, made to volume, and the pectic materials precipitated from an aliquot as calcium pectate as described by CARRÉ-HAYNES (**2**). The calcium pectate gels were found to contain impurities which were largely proteinaceous. The amount of impurities was estimated by determining the nitrogen content of a portion of the dried precipitate by the micro-Kjeldahl method and by calculating the protein content by use of the usual conversion factor, 6.25. This value was subtracted from the weight of the original precipitate to give the amount of calcium pectate.

SOLUBLE PECTIN.—Four grams of the dried plant sample were transferred to a one-liter bottle with 500 ml. of 0.2 per cent. ammonium citrate solution. After shaking for one hour on a mechanical shaker the solution was filtered and a 200-ml. aliquot taken for the determination of the soluble pectic materials, which were determined as described for total pectic material.

PROTOPECTIN.—The percentage of protopectin was calculated by subtracting the percentage of soluble pectin from the percentage of total pectin.

TOTAL NITROGEN.—Portions of the fresh pulp of approximately 5 gm. each were used for total nitrogen by the usual Kjeldahl method. Since no nitrates could be detected the modification to include nitrates was omitted.

EXTRACTION OF NON-PROTEIN NITROGEN.—A sample of 100 gm. of the freshly ground pulp was placed in a mortar and a small quantity of acid washed quartz sand added. The pulp was thoroughly triturated and after the gradual addition of 20 ml. of water the mixture was transferred onto a square of huck toweling suspended over a 2-liter beaker and the extract expressed by hand. The residue was returned to the mortar and the extraction repeated two additional times. The extract was centrifuged to remove as much starch as possible. It was heated to boiling, a few ml. of 5 per cent. Fe_2O_3 solution were added, and the boiling continued for approximately 2 minutes. The solution was filtered in a Buchner funnel containing an asbestos mat. The beaker and the mat were thoroughly washed with hot water. The filtrate was made to a volume of 1000 ml. and preserved with toluene when not analyzed immediately.

TOTAL NON-PROTEIN NITROGEN.—Aliquots of the non-protein filtrate were analyzed as described for total nitrogen.

TOTAL ALPHA-AMINO NITROGEN.—A special preliminary procedure as perfected by STUART and APPLEMAN (**7**) was adopted to eliminate errors that occur in the usual Van Slyke procedure. Aliquots of 10 ml. each were used for the determination of total alpha-amino nitrogen in the Van Slyke apparatus. The volume of the gaseous nitrogen was reduced to standard conditions and the proper corrections made for the blank determinations.

BASIC NITROGEN.—Aliquots of 100 ml. of the non-protein extract were acidified with 2.5 ml. of concentrated sulphuric acid. When cooled to room temperature, 30 ml. of phosphotungstic acid solution (20 gm. phosphotungstic acid and 5 gm. of sulphuric acid made to 100 ml. of solution) were added. After standing for 24 hours in a refrigerator, the solutions were filtered and the precipitates washed thoroughly with a dilute solution of phosphotungstic acid, containing 2.5 gm. of phosphotungstic acid and 5 gm. of sulphuric acid per 100 ml. of solution. The filter paper and the precipitates were transferred to a Kjeldahl flask and digested as for total nitrogen. The ammonia was distilled into 0.01 N acid and the excess acid titrated with 0.01 N base.

NON-AMINO NITROGEN.—The filtrates from the basic nitrogen samples were neutralized with concentrated sodium hydroxide. The solution was then subjected to the distillation treatment and the mono-amino nitrogen was determined in the Van Slyke apparatus.

AMIDE NITROGEN.—Aliquots of 50 ml. of the non-protein extract were hydrolyzed under reflux condensers with 3 ml. of concentrated sulphuric acid in a boiling water bath for 2.5 hours. The solutions were filtrated, the residue washed with a few ml. of dilute sulphuric acid, and the filtrate neutralized with sodium hydroxide. The ammonia was determined by the SESSIONS and SHIVE method (**6**).

HUMIN NITROGEN.—The residues filtered off following the amide hydrolysis were placed in a Kjeldahl flask and digested as for total nitrogen and the ammonia distillation was carried out as for basic nitrogen.

RESIDUAL NITROGEN.—The residual nitrogen was determined by finding the difference between the total non-protein nitrogen and the sum of the determined soluble nitrogen fractions. In the lots cured at 86° and 95° F. with low humidity, the residual nitrogen represents the difference between the total non-protein nitrogen and the sum of the total alpha-amino, amide, and ammonia nitrogen. In all other lots it represents the difference between the total non-protein nitrogen and the sum of the mono-amino, amide, basic, ammonia, and humin nitrogen.

Since there was considerable loss in total weight of sweet potatoes during curing and storage, all of the results are presented as percentages of the fresh weight at the time of harvest in order to have the percentages reveal actual changes in the constituents determined.

Results

Changes in pectic substances

The changes in the pectic substances during curing and storage are shown in table I.

TABLE I

CHANGES IN THE PECTIC CONSTITUENTS IN SWEET POTATOES DURING CURING AND STORAGE. THE RESULTS ARE EXPRESSED AS PERCENTAGES OF FRESH WEIGHT AT HARVEST TIME

Treatment	Date of analysis	Soluble pectin	Protopectin
Cured at 86° F. and low humidity			
		%	%
Curing period	Oct. 12	0.399	0.409
	Oct. 22	0.326	0.532
Cured at 86° F. and 80 to 85% humidity			
Curing period	Oct. 7	0.426	0.350
	Oct. 13	0.428	0.375
	Oct. 18	0.574	0.302
Storage period	Nov. 24	0.535	0.411
	Jan. 1	0.298	0.639
	Feb. 22	0.339	0.654
Cured at 86° F. and 95 to 100% humidity			
Curing period	Oct. 7	0.426	0.350
	Oct. 18	0.538	0.309
Storage period	Nov. 24	0.465	0.548
	Feb. 22	0.372	0.717
Cured at 95° F. and low humidity			
Curing period	Oct. 12	0.399	0.409
	Oct. 22	0.425	0.551
Cured at 104° F. and 70 to 75% humidity			
Curing period	Oct. 7	0.426	0.350
	Oct. 13	0.445	0.465
	Oct. 18	0.582	0.294
Storage period	Nov. 24	0.458	0.377
	Jan. 1	0.252	0.670
	Feb. 22	0.302	0.759
Cured at 104° F. and 90 to 95% humidity			
Curing period	Oct. 7	0.426	0.350
	Oct. 13	0.442	0.414
	Oct. 18	0.598	0.255
Storage period	Nov. 24	0.483	0.554

In all of the lots except the one cured at 86° F. and low humidity, the percentage of soluble pectin in the sweet potatoes increased during curing. The protopectin showed a corresponding decrease. The rate of this transformation was somewhat greater at the higher temperatures. At the storage temperature the protopectin increased again while the pectin decreased. Some of the increase in protopectin was due to an actual increase in the total pectic substances. This storage synthesis of protopectin continued in the roots as long as they remained alive and sound.

Nitrogen metabolism

In table II are shown the changes in the nitrogenous fractions in sweet potatoes during curing and storage. At the time of harvest the total

TABLE II

Changes in the distribution of nitrogen in sweet potatoes during curing and storage. Results are expressed as percentages of fresh weight at harvest time

Treatment	Date of analysis	Total N	Protein N	Non-protein N	Alpha amino N	Amide N	Basic N	Residual N
			Cured at 86° F. and low humidity					
		%	%	%	%	%	%	%
Curing period	Oct. 12	0.217	0.145	0.072	0.047	0.008	0.010	0.016
	Oct. 22	0.223	0.139	0.084	0.044	0.006	0.014	0.032
Storage period	Nov. 23	0.235	0.148	0.087	0.028	0.009	0.020	0.049
	Dec. 31	0.210	0.131	0.079	0.024	0.015	0.017	0.038
			Cured at 86° F. and 80 to 85% humidity					
Curing period	Oct. 7	0.190	0.149	0.041	0.029	0.0015	0.008	0.012
	Oct. 13	0.187	0.143	0.044	0.041	0.0021	0.009	0.011
	Oct. 18	0.162	0.116	0.046	0.025	0.0033	0.008	0.017
Storage period	Nov. 24	0.157	0.102	0.055	0.030	0.0029	0.014	0.015
	Jan. 1	0.145	0.098	0.047	0.026	0.0025	0.016	0.008
	Feb. 22	0.177	0.099	0.078	0.043	0.0063	0.016	0.026
			Cured at 86° F. and 95 to 100% humidity					
Curing period	Oct. 7	0.190	0.149	0.041	0.029	0.0015	0.008	0.012
	Oct. 18	0.189	0.132	0.057	0.030	0.0036	0.013	0.020
Storage period	Nov. 24	0.184	0.127	0.057	0.034	0.0040	0.011	0.011
	Feb. 22	0.162	0.113	0.049	0.026	0.0026	0.024	0.002
			Cured at 95° F. and low humidity					
Curing period	Oct. 12	0.217	0.145	0.072	0.047	0.008	0.010	0.016
	Oct. 22	0.250	0.150	0.100	0.061	0.002	0.022	0.036
Storage period	Dec. 10	0.207	0.113	0.094	0.025	0.016	0.017	0.051
	Feb. 10	0.208	0.118	0.120	0.013	0.025	0.022	0.090
			Cured at 104° F. and 70 to 75% humidity					
Curing period	Oct. 7	0.190	0.149	0.041	0.029	0.0015	0.008	0.012
	Oct. 13	0.159	0.095	0.064	0.049	0.0049	0.008	0.013
	Oct. 18	0.175	0.088	0.087	0.058	0.0086	0.013	0.019
Storage period	Nov. 24	0.185	0.109	0.076	0.040	0.0117	0.015	0.017
	Jan. 1	0.176	0.089	0.087	0.048	0.0071	0.022	0.013
	Feb. 22	0.178	0.083	0.095	0.049	0.0158	0.020	0.022
			Cured at 104° F. and 90 to 95% humidity					
Curing period	Oct. 7	0.190	0.149	0.041	0.029	0.0015	0.008	0.012
	Oct. 18	0.141	0.056	0.085	0.155	0.0084	0.013	0.018
Storage period	Nov. 24	0.164	0.091	0.073	0.041	0.0089	0.013	0.018
	Feb. 22	0.170	0.057	0.103	0.051	0.0169	0.020	0.022

organic nitrogen in the roots averaged only 0.2 per cent. of the fresh weight, or 0.8 per cent. of the total solids. The non-protein nitrogen in the roots at harvest time in percentage of total nitrogen was 21.58 per cent. in one

crop and 33.18 in another crop. The non-protein nitrogen increased in all lots during curing. At the same time the protein nitrogen decreased, indicating protein hydrolysis during curing. The rate of hydrolysis was somewhat higher at the higher curing temperatures. There were consistent increases in the basic and residual nitrogen at all curing temperatures, but consistent increases in the amino and amide nitrogen occurred only at high curing temperature.

The nitrogen distribution in the sweet potatoes during storage at 50° to 53° F. remained fairly stable during most of the storage period but there was definite evidence of slight protein hydrolysis near the end of the storage period, especially in the lots cured at the highest temperature. The slight changes in the amino nitrogen during the entire storage period are of doubt-

TABLE III

DISTRIBUTION OF NITROGEN IN THE PROXIMAL AND DISTAL HALVES OF SWEET POTATOES. RESULTS ARE EXPRESSED AS PERCENTAGES OF THE FRESH WEIGHT AT THE TIME OF ANALYSIS

	Moisture	Total N	Non-protein N	Alpha amino N	Amide N	Basic N	Residual N
Harvest sample, October 7							
	%	%	%	%	%	%	%
Proximal half	75.68	0.187	0.044	0.020	0.003	0.010	0.017
Distal half	75.60	0.181	0.044	0.019	0.003	0.010	0.018
Potatoes cured and stored until November 27							
Proximal half	75.84	0.178	0.062	0.036	0.008	0.010	0.025
Distal half	75.81	0.172	0.054	0.032	0.007	0.013	0.013

ful significance but the amide, basic, and residual nitrogens showed significant increases in most lots near the end of the storage period. These nitrogen fractions were also calculated as percentages of total nitrogen and these percentages definitely confirm the changes indicated in table II. The ammonia and humin nitrogens were determined but the results are not included in the tables as the amounts were very small and they showed no definite trends during curing or storage. The mono-amino acid nitrogen is not included in the tables as the changes in this fraction correspond almost exactly with those of the alpha amino acid, the absolute quantities are less by the alpha amino nitrogen in the basic fraction.

DISTRIBUTION OF NITROGENOUS COMPOUNDS IN THE PROXIMAL AND DISTAL ENDS OF THE SWEET POTATO

Analyses were made of the proximal and distal halves of the harvest sample and of potatoes that had been in storage for six weeks. The samples

were extracted on the dates indicated in the table. The non-protein nitrogen solution was preserved with toluene, stored at room temperature, and analyzed on February 9. The results are given in table III.

The proximal half showed a slightly higher total nitrogen content. The variations were so small that it may be concluded there is no apparent difference in the nitrogen distribution in the proximal and distal halves of the sweet potato.

Summary

A study was made of the nitrogen metabolism and the pectic transformations in Maryland Golden Sweet Potatoes during curing under different combinations of temperature and humidity and also during subsequent storage.

The soluble pectin in the sweet potatoes increased during curing. The protopectin showed a corresponding decrease. An increase in the total pectic material occurred in all lots.

At the storage temperature the protopectin increased again while the pectin decreased. Some of the increase in protopectin was due to an actual increase of the total pectic substances. This storage synthesis of protopectin continued in the roots as long as they remained alive and sound.

The non-protein nitrogen in the sweet potatoes at harvest time ranged from 22 to 33 per cent. of the total nitrogen. Under all of the curing conditions the non-protein nitrogen increased and the protein nitrogen decreased indicating protein hydrolysis. The rate of this hydrolysis was somewhat higher at the higher curing temperatures. Fairly consistent increases in basic, amide, and residual nitrogen occurred in all lots but a consistent increase in amino nitrogen occurred only in those lots cured at the high temperatures of 95° and 104° F.

The nitrogen distribution in the sweet potatoes during storage at 50° to 53° F. remained fairly stable during most of the storage period. The amide, basic, and residual nitrogens increased slightly in most lots near the end of the storage period.

There was no apparent difference in the nitrogen distribution in the proximal and distal halves of the Maryland Golden Sweet Potato.

A temperature of 86° F. for curing the Maryland Golden Sweet Potato, gave the best results in storage. Higher temperatures were unsuitable because of internal break-down during curing. At 86° F. a high relative humidity was the decisive factor for effective curing of this variety, but there was no conclusive evidence that humidity had any effect on the nitrogen metabolism in the roots during curing.

The curing of sweet potatoes appears to involve a number of interrelated internal processes. Studies to date do not reveal any one outstanding essential change in the potatoes during curing.

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SOME EFFECTS OF SODIUM SALTS ON THE GROWTH OF THE TOMATO[1]

H. E. Hayward and E. M. Long
(with six figures)

Introduction

In a previous study (**6**) the influence of high osmotic concentrations of sodium salts and nutrient solutions upon the vegetative development of the tomato was reported, but no data were obtained on the fruiting responses. To investigate this problem, to obtain further information on the relative toxicity of the Cl^- and $SO_4^=$ ions, and the effect of osmotic concentration, experiments were set up to determine the response of tomato plants to sodium chloride and sodium sulphate when supplied together in different proportions and at several levels of total concentration.

Experimental procedure and methods

Marglobe tomatoes were grown under greenhouse conditions in automatically irrigated sand cultures of the type designed by Eaton (**2**). Two series of five treatments each were set up with three replications. The treatments were: Base nutrient solution (control); and base nutrient + 40, 80, 120, and

TABLE I

Constitution of culture solutions

Series and culture	Treatment	Osmotic conc.	Salts					
			$Ca(NO_3)_2$	KNO_3	$MgSO_4$	KH_2PO_4	NaCl	Na_2SO_4
	m.e. Na/l.	*atm.*	*m.e./l.*	*m.e./l.*	*m.e./l.*	*m.e./l.*	*m.e./l.*	*m.e./l.*
A & B–1	Control	1.6	14.4	14.4	14.4	1.8	.....	.....
A–2	40	2.9	"	"	"	"	10	30
A–3	80	4.1	"	"	"	"	20	60
A–4	120	5.3	"	"	"	"	30	90
A–5	160	6.4	"	"	"	"	40	120
B–2	40	3.2	"	"	"	"	30	10
B–3	80	4.6	"	"	"	"	60	20
B–4	120	6.1	"	"	"	"	90	30
B–5	160	7.7	"	"	"	"	120	40

Micro-nutrients: B, 1 p.p.m.; Zn, 0.1 p.p.m.; Mo, 0.1 p.p.m.; Mn, 0.4 p.p.m.; Cu, 0.01 p.p.m.; Fe, 5 p.p.m., supplied as iron citrate.

Analysis of Riverside tap water, m.e./l.

Ca	Mg	Na	K	Cl	SO_4	HCO_3
1.40	0.23	1.51	0.05	0.45	0.75	2.50

[1] Contribution from the U.S. Regional Salinity Laboratory, Bureau of Plant Industry, Soils, and Agricultural Engineering, Riverside, California in cooperation with the eleven western states and the Territory of Hawaii.

160 milliequivalents per liter of sodium salts. To segregate the supplementary effects of the anions, 25 per cent. of the sodium was supplied as Cl^- and 75 per cent. as $SO_4^=$ in series A; and 75 per cent. as Cl^- and 25 per cent. as $SO_4^=$ in series B. The constituents of the base nutrient solution, the amounts of sodium salts added, the osmotic concentration of the culture solutions, and the analysis of the tap water at Riverside, California, are shown in table I.

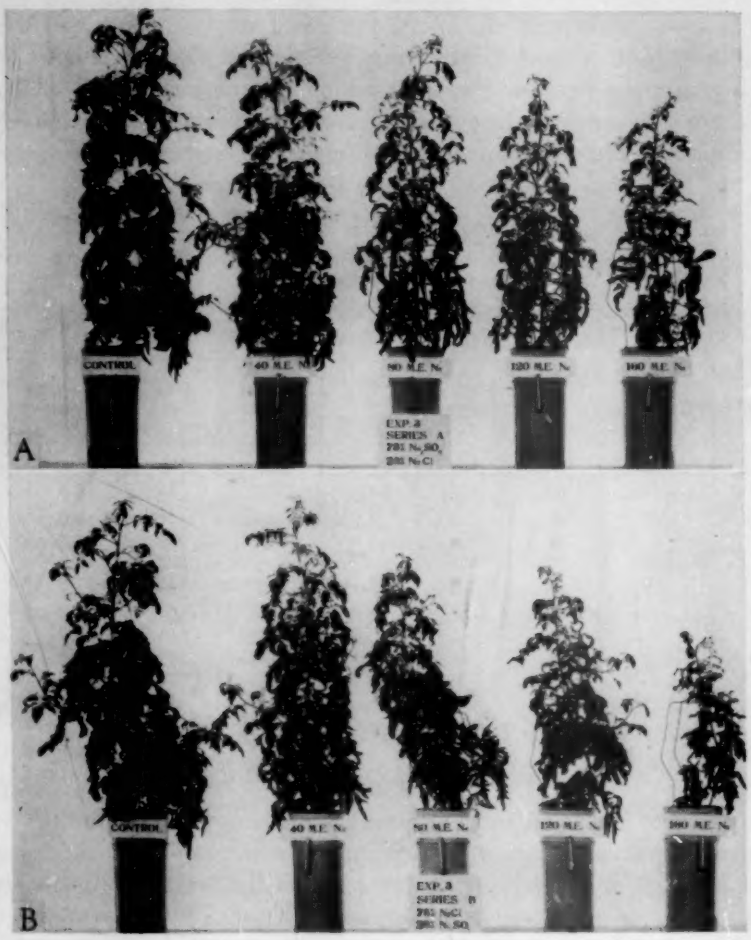

FIG. 1. Growth response to treatment with sodium salts. A. Series A (high sulphate treatments); B. Series B (high chloride treatments). The osmotic concentration for each sodium treatment is shown in figure 6. Photographed five weeks before the final harvest.

All cultures were started with the base nutrient solution; and, after the seedlings were well established, sodium salts were added by 20-m.e. increments at two-day intervals to bring the respective treatments to full concentration. Seven changes of the solutions were made during the course of the

experiment to maintain the osmotic concentration within a 0.5 atm. range and to keep the nutrient ions above deficiency levels. The H-ion concentration was adjusted daily to a pH of 7.0 with HNO_3. The volume of the culture solutions was maintained by the addition of tap water daily, or twice daily when transpiration was high.

Planting, thinning, and harvest dates

The seeds were planted July 8, 1940, and six plants per culture were maintained until August 6, when four were harvested. On August 28, one of the plants was harvested, and the remaining one was carried to the final harvest on November 8. To conserve space and permit a better analysis of flowering and fruit development, the vines were pruned to single stems (fig. 1).

TABLE II

Fresh and dry weights, percentage of dry matter, and osmotic concentration of expressed juice of vines

Series and culture	Treatment	Height	Harvest, 8–28–40		Final harvest, 11–8–40			
			Fresh wt.*	Osmotic conc. juice	Fresh wt.*	Dry wt.	Dry matter	Osmotic conc. juice
	m.e. Na/l.	*cm.*	*gm.*	*atm.*	*gm.*	*gm.*	*%*	*atm.*
A & B–1	Control	65	494	10.7	2155	353	16.4	11.6
A–2	40	67	343	11.2	1910	327	16.9	12.4
A–3	80	60	244	11.9	1664	285	17.1	13.6
A–4	120	51	151	12.7	1180	204	17.3	15.0
A–5	160	46	104	13.7	797	142	17.8	15.0
B–2	40	64	310	12.6	1832	324	17.7	13.7
B–3	80	57	206	12.9	1662	261	15.7	14.9
B–4	120	45	117	13.3	1091	181	16.6	16.4
B–5	160	35	74	15.3	443	75	17.0	15.9

* All weights are averages of tops per plant.

Methods of analysis of juice

In order to determine to what extent the ions were accumulating, and to get some indication of the effect of the salt treatments on carbohydrate synthesis, analyses of the juice of the tops and of the fruit were made for the principal ions, reducing sugar, and organic nitrogen. Samples of vegetative material and fruit were rapidly frozen with dry ice and placed in cold storage until analyses could be made. The material was then thawed, the juice expressed with a Carver press under 2000 pounds pressure per square inch, and the freezing point depression of the juice determined. The juice was centrifuged for 15 minutes at 1800 r.p.m., hydrogen-ion concentrations determined, and chemical analyses made by A.O.A.C. methods except for K^+ which was assayed by the Hibbard and Stout procedure (**7**). Analyses were run for reducing sugars. Non-reducing sugars were not found in significant quantities. The sap was cleared by the method described by Hassid

(5), and the sugar determinations were made by a modified HARDING and DOWNS procedure as outlined by VAN DER PLANK (12).

Results

VEGETATIVE RESPONSES AND GROWTH DATA

Growth data were obtained at the intermediate and final harvests. The higher the concentration of the culture solution, the greater was the reduc-

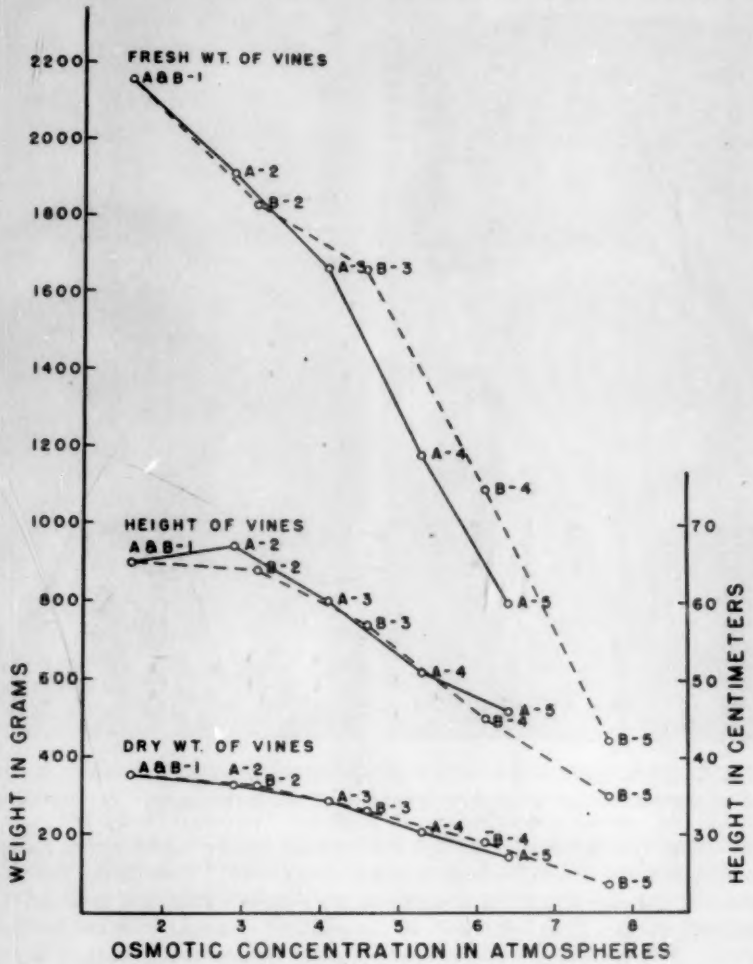

FIG. 2. Height, and fresh and dry weight of vines, plotted against osmotic concentration of the substrate. The letters A and B refer to salt treatments as shown in table I. Series A, high sulphate treatments; and series B, high chloride treatments.

tion in the height of stems and fresh and dry weight of vines (table II). At equivalent concentration of sodium, plants in the high chloride series showed greater growth inhibition than those in the high sulphate series (figs. 1

and 4). This might suggest that the toxic effect of the Cl^- ion exceeds that of the $SO_4^=$ ion, but it should be recognized that the activity of the Cl^- ion is much higher than that of the $SO_4^=$ ion in the respective solutions. Osmotic concentration is possibly the dominant factor in this difference in growth response since, at corresponding levels of sodium treatment, the osmotic concentration of the culture solution is greater in the chloride series, the

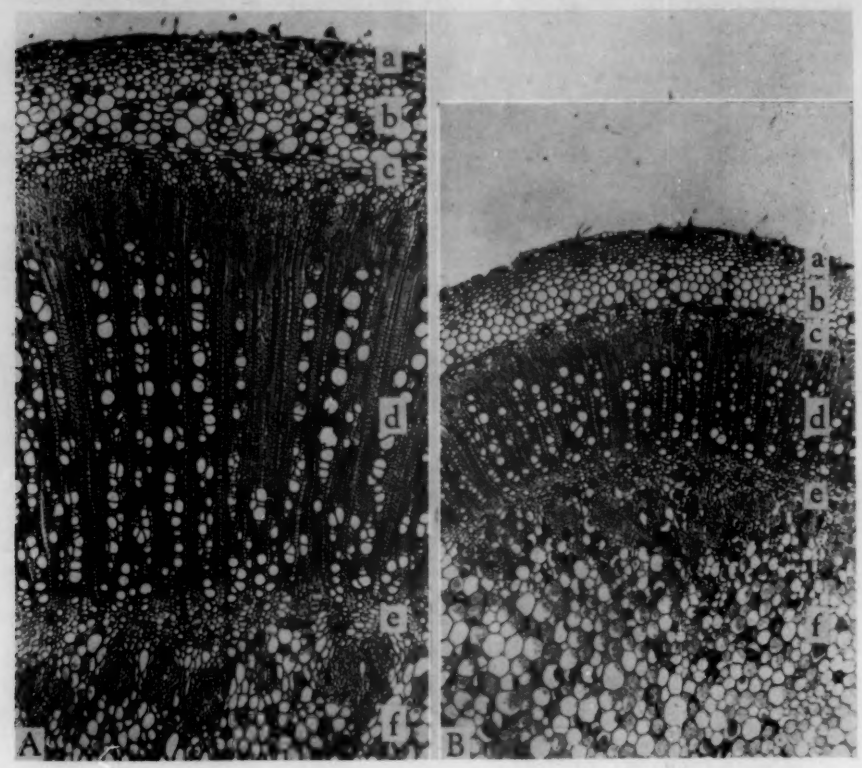

FIG. 3. Histological responses to high concentrations of sodium salts. Comparable sectors of transections of the middle internode of the tomato vine. A. Control culture (A–1) 1.6 atm. osmotic concentration; B. High salt treatment (A–5) 160 m.e. Na/l., 6.4 atm. osmotic concentration. The high salt stem has smaller, thicker-walled collenchyma (a), pericyclic fibers (c), and secondary xylem elements (d). The parenchymatous cells of the cortex (b) and pith (f) are smaller in the high salt stem, and those of the pith contain more starch. (The larger cells of the pith in the control stem are not shown in the figure.) The smaller size of the zone of secondary vascular tissues in B, from pericycle (c) to primary xylem (e), is the result of a slower rate of cambial activity at high osmotic concentrations, and the maturation of cells of smaller size. A and B at same magnification.

differences ranging from 0.3 atm. with 40 m.e. of added salt to 1.3 atm. at the 160 m.e. level (table I). When height of stems and fresh and dry weight of vines are plotted against the osmotic concentration of the culture solutions, the differential effect of the Cl^- and $SO_4^=$ ions is very small (fig. 2). In fact, at isosmotic concentrations, there is little difference in the growth inhibition as expressed in terms of height of stems and dry weight of vines,

and the chloride plants (series B) had greater fresh weights of vines than did the sulphate plants (series A) at the higher levels. This is probably due to the tendency of plants to be more succulent when grown in high chloride than in high sulphate solutions.

Anatomical responses

The anatomical and histological responses observed were similar to those noted in an earlier study with high concentrations obtained by additions of sodium salts to a base nutrient solution and by multiplying the constituents

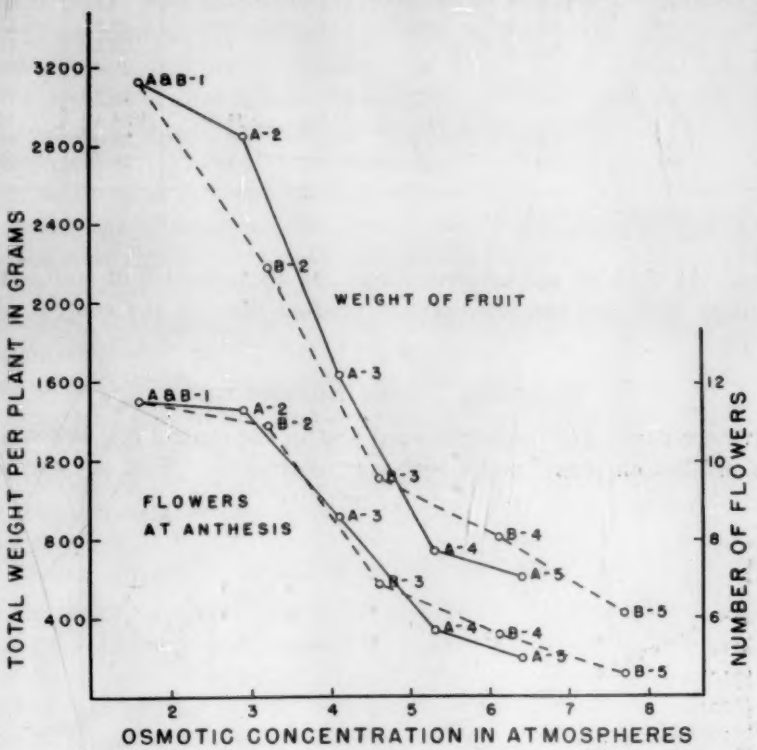

Fig. 4. Number of flowers at anthesis, daily average for 30 days, and total weight of fruit per plant plotted against osmotic concentration of the substate. The letters A and B refer to salt treatments as shown in table I. Series A, high sulphate treatments; and series B, high chloride treatments.

of the base nutrient (6). The smaller stems of vines grown at the high sodium levels as compared with the control vines were the result of decreased cambial activity, consequent reduction in the amount of secondary vascular tissue differentiated, and the smaller size of the mature cells. This was especially noticeable in the secondary xylem vessels and cells of the mechanical tissues of the high salt plants which were much smaller and had proportionately thicker walls. There was also a marked reduction in the amount of cortical and medullary parenchyma and in the size of the cells of those

TABLE III

FLOWERING AND FRUITING RESPONSES

SERIES AND CULTURE	TREATMENT	FLOWERS AT ANTHESIS*	FRUIT SET†	FRUIT HARVESTED			
				NO. PER PLANT	TOTAL WT. FRUIT PER PLANT	WT. PER FRUIT	BLOSSOM-END-ROT
	m.e. Na/l.				*gm.*	*gm.*	*%*
A & B–1	Control	11.5	188.5	56.5	3124	55.2	16.8
A–2	40	11.3	207	57.0	2856	50.1	33.3
A–3	80	8.6	136	55.7	1638	29.4	39.5
A–4	120	5.7	72	33.7	743	22.1	18.8
A–5	160	5.0	73	30.0	615	20.5	36.7
B–2	40	10.9	197	55.7	2183	39.2	29.9
B–3	80	6.9	111	39.3	1115	28.4	44.1
B–4	120	5.6	72	34.0	816	24.0	41.2
B–5	160	4.6	28	16.0	427	26.7	18.8

* Daily average, 30 days.
† Average per replication.

tissues. At the high salt concentrations, the accumulation of starch in the medullary and ray parenchyma was greater than in the control plants (fig. 3).

FLOWERING AND FRUITING RESPONSES

Evident flower buds were observed first in the control and low salt cultures and last on plants under high salt treatments. With one exception,

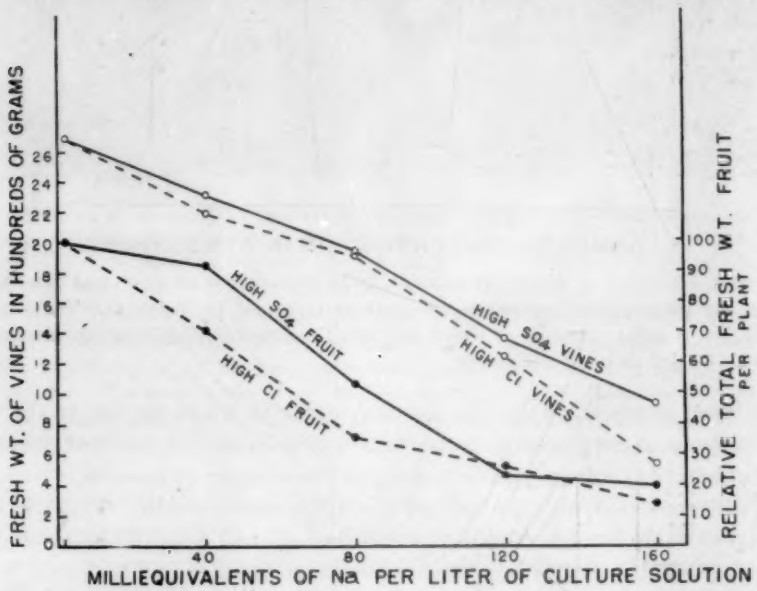
FRESH WT. OF VINES IN HUNDREDS OF GRAMS
RELATIVE TOTAL FRESH WT. FRUIT PER PLANT
HIGH SO4 VINES
HIGH CI VINES
HIGH SO4 FRUIT
HIGH CI FRUIT
MILLIEQUIVALENTS OF Na PER LITER OF CULTURE SOLUTION

FIG. 5. The fresh weight of the vines and the relative total fresh weight of the fruit per plant for series A (high sulphate treatments) and series B (high chloride treatments).

this sequence also obtained for anthesis, the lag ranging from 3 to 6 days. From August 20 to September 24, floral counts were made to determine daily averages of flowers at anthesis per plant under each treatment. In both series, there was a marked decrease in the number of flowers formed with increasing concentrations of salt, but there was no significant difference in the effect of the Cl^- and $SO_4^=$ ions (fig. 4). The total weight of fruit per plant and weight per fruit were reduced by the salt treatments. The reduction in total weight of fruit was not great at the 40 m.e. level in series A, but there was a sharp drop at higher salt concentrations (table III). The crop at the 120 m.e. level was about 25 per cent. of that of the control, and less than 15 per cent. at the 160 m.e. salt level in the B series (high chloride) (fig. 5).

On the basis of osmotic concentration, the high sulphate plants (series A) produced a greater total weight of fruit per plant than those of the high chloride series (B) at the lower osmotic values, but the reverse was true at the upper levels of salt concentration (fig. 4). Like the vegetative responses, the production of fruit indicates that the osmotic concentration of the substrate is more significant than the specific effect of the Cl^- and $SO_4^=$ ions.

The incidence of blossom-end rot was high in all treatments. ROBBINS (11) found that approximately 80 per cent. of fruits on plants grown with solutions adjusted to 1.7 and 3.1 atm. osmotic concentration developed blossom-end rot and suggested that it was associated with wide fluctuations in the rates of evaporation, and occurred when the rate of transpiration was high. We observed that it was possible to reduce the amount of blossom-end rot by bringing the solutions up to volume frequently, thereby reducing the variations in water stress.

ANALYSIS OF JUICE OF VINES

The osmotic concentration of the juice of the vines increased as the vines matured. In every case, the juice of vines harvested in November was more concentrated than that for equivalent plants harvested in late August. At the final harvest, the osmotic concentration of the juice was greater at high concentrations of the culture solution, except at the highest levels where there was little change or a slight reduction as compared with the next lower level of salt treatment. Equivalent concentrations of sodium resulted in a higher osmotic concentration in the sap in the chloride than in the sulphate series, following the relationship of the culture solutions in this respect (fig. 6).

Calcium, magnesium, and potassium were supplied to all cultures in equal amounts, sodium being progressively increased from 1.6 m.e./l. (average content in Riverside tap water) to 160 m.e./l. In general, the total cations present in the juice tended to be uniform regardless of treatment. With one exception (B–5), there was an increase in sodium at each succeeding higher sodium treatment and this was accompanied by a decrease in the

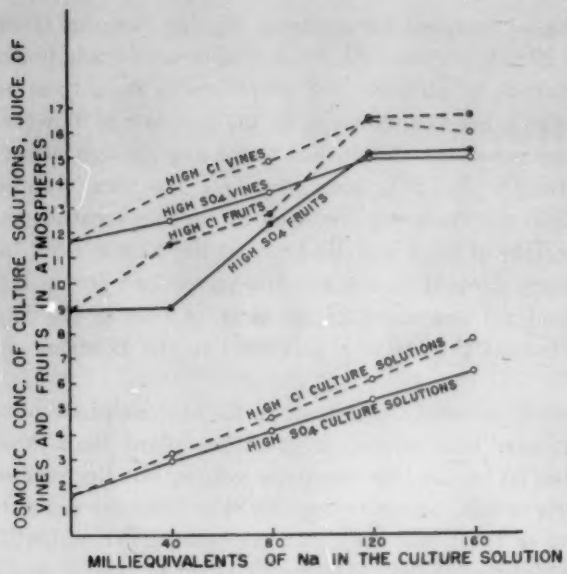

FIG. 6. The osmotic concentrations for the culture solutions, juice of vines, and juice of fruit at the different levels of sodium treatment. The high sulphate curves refer to series A, high chloride curves to series B.

concentration of other cations in the juice. In the A series (high sulphate) with increasing increments of sodium salts, the reduction in the amounts of Ca, Mg, and K was progressive. In the B series (high chloride), the relationship was not so clear owing to anomalous results from the B–3 cultures. With one exception (B–5), chloride and sulphate increased in the vines with increasing concentration in the culture solutions, the proportion of the two sodium salts in them being reflected in the greater SO_4 accumulation in the vines of the A series and the increased Cl concentration in those of the series B (table IV).

TABLE IV

ANALYSIS OF JUICE OF TOPS

SERIES AND CULTURE	TREATMENT	Ca	Mg	Na	K	SO_4	Cl	N*	REDUCING SUGAR
	m.e. Na/l.	*m.e./l.*	*m.e./l.*	*m.e./l.*	*m.e./l.*	*m.e./l.*	*m.e./l.*	*m.e./l.*	*%*
A & B–1	Control	81	156	10	163	113	13	92	1.42
A–2	40	97	140	55	135	136	41	83	1.24
A–3	80	69	139	110	115	164	65	96	1.20
A–4	120	51	130	135	117	171	92	117	1.17
A–5	160	48	111	151	112	174	92	130	1.21
B–2	40	85	158	75	120	118	102	104	1.47
B–3	80	50	115	121	101	108	162	95	1.00
B–4	120	70	127	150	110	122	184	112	1.17
B–5	160	70	128	114	112	120	168	111	1.11

* Organic and ammoniacal N.

TABLE V

ANALYSIS OF JUICE OF RIPE FRUIT. OSMOTIC CONCENTRATION, ACCUMULATION OF IONS, N, AND PERCENTAGE N, REDUCING SUGAR AND ACID

SERIES AND CULTURE	TREATMENT	OSMOTIC CONC.	Ca	Mg	Na	K	SO_4	Cl	N*	NITROGEN	REDUCING SUGAR	ACID†
	m.e. Na/l.	*atm.*	*m.e./l.*	*m.e./l.*	*m.e./l.*	*m.e./l.*	*m.e./l.*	*m.e./l.*	*m.e./l.*	%	%	%
A & B–1	Control	8.9	1.4	8.2	6.9	69.2	4.5	3.5	99	0.14	3.35	0.52
A–2	40	9.0	1.4	7.7	5.5	64.6	5.6	6.9	81	0.11	3.88	0.53
A–3	80	12.3	2.0	9.3	6.6	77.5	7.6	10.5	106	0.15	5.38	0.61
A–4	120	15.1	1.4	10.1	8.7	108.2	9.7	14.2	158	0.22	6.19	0.82
A–5	160	15.2	4.5	17.5	11.5	115.8	11.1	18.2	179	0.25	5.78	0.88
B–2	40	11.5	1.3	9.1	6.5	80.3	5.2	13.4	110	0.15	4.06	0.62
B–3	80	12.7	2.4	11.3	7.1	92.4	5.7	18.4	125	0.17	4.70	0.63
B–4	120	16.5	7.1	14.4	12.4	119.4	9.5	23.7	206	0.29	5.73	0.84
B–5	160	16.6	7.0	17.1	12.7	121.2	8.5	31.5	209	0.29	5.33	0.86

* Organic and ammoniacal N.
† Calculated as citric acid.

Organic nitrogen increased in plants grown under high sodium treatments, but there was little or no increase at intermediate levels. Reducing sugars were lower in the salt treatments than in the controls except in the B–2 culture, but the differences between treatments were slight (table IV).

Analysis of juice of ripe fruit

In ripe fruits, the osmotic concentration of the expressed juice increased with increasing concentration of the sodium salts in the nutrient solution; and, like the vines, the osmotic concentrations were higher in the B series at corresponding levels of treatment. As Chandler (1) and Robbins (11) have pointed out, in most cases the osmotic concentration of the juice of vegetative parts tends to exceed that of the fruit. At control, low, and intermediate levels of sodium concentration where the osmotic concentration of the substrate ranged from 1.6 to 4.6 atm. we found that the osmotic concentration of the vegetative sap exceeded that of the fruit by one to three atm. At the higher levels of salt treatment, however, in which the osmotic concentrations of the culture solutions were from 5.3 to 7.7 atm., the osmotic concentration of the fruit juice equaled or exceeded that of the vegetative sap (fig. 6).

These relationships suggest that hydrostatic stresses may be operating to limit hydration which, as MacDougal (10) has pointed out, is fundamental to growth. It seems probable that the growth of fruit at the highest salt levels was inhibited not so much by lack of food reserves as by inadequate hydration. As indicated in table V, the organic nitrogen in the juice of the fruit increased progressively up to the highest level of salt treatment; and the percentage of reducing sugar was highest under the 120 m.e. Na/l. treatment and only slightly less than that peak under the highest salt concentrations.

As compared with the vegetative tissues, the accumulation of Ca, Mg, Na, SO_4, and Cl in the fruit was low, and differences as the result of treatment were small (table V). Potassium and nitrogen occurred in larger amounts, the former approximating the accumulation in the vegetative saps at the high salt levels. With one exception, nitrogen values of the fruit juices were progressively higher with increasing concentration. The accumulation of sodium in the juice of the fruit increased with increasing increments of sodium in the substrate except for a slight decrease in the A–2 culture. The accumulations of SO_4 and Cl reflected the treatments, more of the former occurring in the A series, and of the latter in the B; but the amounts present were small as compared with the vegetative juice and with the concentration of these ions in the substrates in which the plants were grown (table V).

Discussion

Analyses of the fruit juices indicated that on a percentage basis, there was a slight increase in nitrogen, reducing sugars and mineral constituents with increasing concentrations of salts. Flavor and quality may be ad-

versely affected by the presence of Cl, SO_4, and Na ions and by the higher acid values that were found under high salt treatments, but the amounts of these ions accumulated as compared with their concentration in the vegetative sap were small. It should be added that no determinations were made for vitamin content of the fruit, such as those of HAMNER, LYON and HAMNER (**4**) for ascorbic acid, so that we have no information as to how high salt treatment may affect that aspect of the nutritional picture.

The principal deleterious effects of high salt concentration under the conditions of this study appear to be marked reduction in the number of fruits set and the weight and size of the fruits that reach maturity. Studies with several varieties of tomatoes yielding results in line with those given here have been reported by other investigators. ROBBINS (**11**) experimenting with the same variety used in our work, found that fruits produced on plants grown in nutrient solutions of 3.1 atm. osmotic concentration were much smaller than those grown at lower concentrations. LYON (**8**) using added Na_2SO_4 at 80 and 120 m.e./l. levels, found significant reductions in the mean weight of tomatoes of the Johannisfeuer variety and a reduction of 40 per cent. in the fresh weight of fruit produced at the higher salt level. EATON (**3**) growing the Stone variety at two concentrations of chloride and sulphate salts (50 and 150 m.e./l.) obtained 19 per cent. and 96 per cent. reductions in the relative dry weights of fruits at the low and high chloride levels respectively, and a 28 per cent. and 73 per cent. reduction with the corresponding sulphate treatments.

There also appears to be an increase in the incidence of blossom-end rot when the osmotic concentration of the substrate is high, although this condition is somewhat alleviated when fluctuations in water stress are reduced by keeping the concentration of the solution constant by frequent additions of water. Although our experience, like that of ROBBINS (**11**), indicates that irregularities in hydrostatic stress and the restriction of rate of absorption of water are probably the most important factors in the development of blossom-end rot, there is some evidence that the lack of accumulation, or the accumulation in excess, of certain ions may be contributing causes of this disorder.

On the basis of work reported here and additional unpublished data, it appears that the accumulation of potassium may be significant in blossom-end rot and that calcium may have an ameliorating effect. LYON, BEESON and BARRENTINE (**9**) using the Bonny Best variety and working with nutrient solutions of relatively low concentration found that "fruits produced in treatments where rot was most severe were low in calcium content and high in potassium and magnesium content." Our data are not directly comparable to those of the workers just mentioned since we were concerned with very high concentrations of total ions and of K, Mg, and Ca, and they were working at much lower levels. EATON (**3**) using Stone tomatoes in sand culture has obtained data that suggest that "calcium and magnesium accumulations singly or combined were important contributing factors" to the incidence of blossom-end rot.

Summary

1. Marglobe tomatoes were grown in sand cultures using a nutrient solution with NaCl and Na_2SO_4 as the added salts. Two series were set up with five treatments each: control, 40, 80, 120, and 160 m.e. Na/l. To segregate supplementary effects of the anions, series A had the sodium supplied as 25 per cent. Cl^- and 75 per cent. $SO_4^=$, series B as 75 per cent. Cl^- and 25 per cent. $SO_4^=$. The plants were grown to maturity to obtain data on flowering and fruit production.

2. The osmotic concentration of the substrate appears to be a primary factor in growth inhibition, although secondary effects of the Cl^- and $SO_4^=$ are noted.

3. The principal effects of high concentration of the substrate were:
   a. Reduction in height and diameter of stems and fresh and dry weight of vines.
   b. Reduction in cambial activity, maturation of cells of smaller size, and relatively thicker walls in xylem elements and mechanical cells.
   c. Inhibited floral development and reduced set of fruit.
   d. Reduction in total yield of fruit and in size and weight per fruit.
   e. Increase in osmotic concentration of the vegetative and fruit juices.

4. The accumulation of Ca, K, Mg, and N in the juice of the ripe fruit was greater at high osmotic concentrations of the substrate although those elements were supplied in equal amounts for all treatments.

5. The accumulation of Cl, SO_4, and Na in the vegetative and fruit juices increased with increasing increments of salt in the substrate.

6. Flavor did not appear to be impaired by salt treatments.

7. The incidence of blossom-end rot appeared to be related to wide fluctuations in water stress. It is suggested that high accumulation of potassium may be a contributing factor.

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STIMULATION OF THE ONION ROOT BY ALTERNATING CURRENT

L. Joe Berry and Rosalie C. Hoyt

(with nine figures)

Introduction

Positive direct current sent up an onion root may polarize or stimulate the root, *i.e.*, increase or decrease, respectively, the positivity of the electric potential of the distal contact measured with respect to the earthed proximal contact. Current sent down the root may depolarize or less commonly stimulate the root, *i.e.*, decrease or increase the positivity of this electric potential. The type of change which the current produces is dependent, at least in part, on the strength and duration of the current; the temperature and oxygen tension of the environment; the previous treatment; the individual characteristics; and the segment of the root under investigation (**2, 3**). Since the flow of direct current in a given direction may result in oppositely oriented potential changes which appear to summate in certain cases, it was believed that the use of alternating current would simplify the observations by eliminating one of the two effects, *i.e.*, the polar effect. With this in mind the present investigation was undertaken and the results indicate that the simplification occurred only in part.

Clark (**5**) found that alternating current (6v) applied to Avena coleoptiles diminished the PD of 25 sections mounted in parallel between two agar blocks to which electrical connection was made. The direction of the change in potential is in agreement with the results reported below but no analysis of the action was attempted. Amlong and Bünning (**1**) and Bünning (**4**) found the threshold value for AC stimulation which resulted in a decrease in the potential difference of roots and stems of several plants. Metzner (**6**) claims to have demonstrated rectification in 50 cycle AC in several tissues, but experimental details are lacking. This report indicates that stimulation with DC or AC elicits apparently identical electrical changes in the root, confirming many of the experimental observations previously described (**3**). Moreover, the environmental factors, oxygen and temperature, as well as the previous treatment of the root, are found to influence irritability.

Method

The apparatus described by Berry and Hoyt (**3**) was used for this investigation. The onion roots were grown in the usual manner and the experimental procedures were unchanged except for the use of alternating current. In experiments where both alternating and direct currents were applied at intervals to the root the DC circuit previously diagrammed (**2**) was employed. The 60-cycle, 110-volt house current was led from a potentiometer and connected to the root through a rotary switch. In series with the root

was a Leeds and Northrup type 2450 astatic dynamometer with telescope and scale. The sensitivity was 0.798 microampere (root mean square) per millimeter on the scale. The 60-cycle voltage applied across the root was a pure sine wave, as tested with a cathode ray oscillograph. The current through the root was also found to be a pure sine wave, with no apparent rectification. Reversal of the AC leads did not affect the results. An RCA Ultrasensitive DC Meter was used for measuring potentials. This meter could be read to 0.5 millivolts (one-fourth scale division) and had a period of 3 seconds. As in the previous papers (**2, 3**), potential differences were not determined during the application of current but were recorded at 30-second intervals at all other times during any experiment. In preparing the curves for publication not all points were included if their omission produced no alteration in the curves.

Results

Effect of alternating current of increasing strength on the potential of various segments of the root

Alternating current of increasing strength was applied for thirty seconds every eight minutes to the first five millimeters of the root tip. The contacts were then raised successively to 3 and 8, 5 and 10, 10 and 15, and back to 0 and 5 millimeters above the tip of the same root. Insets W, X, Y, and Z in figure 1 show the positions of the contacts around the root. Typical results for this procedure are given respectively in curves A, B, C, D, and A′. The first 5 millimeters, curves A and A′, show a decrease in potential following the application of 3.2 microamperes. The magnitude of the response (decrease in potential) increased as the current was increased up to 7.2 microamperes, curve A. A similar response to this current after the other segments of the root had been tested is found in curve A′. With still greater current, 12.8 microamperes, the response was slightly less. This effect is described in more detail in a section below.

In the more basal segments of the root, stimulation occurred only with stronger current; 19.6 microamperes were required in curve B, 12.0 microamperes in curve C and 27.2 microamperes did not stimulate the most basal segment as shown in curve D. These results are in agreement with those reported for direct current (**3**) in which polarizations and depolarizations, rather than stimulations, were usually observed in basal segments. It should be stated, however, that in some roots a small response may sometimes be obtained even in basal segments but always the required current is higher and the magnitude of potential change is smaller than in the apical segment (fig. 8, curve B).

In one root it was found that the potential following the flow of alternating current increased (more positive) rather than decreased. The response of this root is shown in figure 2. Current was applied for 30 seconds. The initial potential of the apical 5 millimeters was about 10 millivolts *negative* (curve A) and thus was oriented opposite to that of the majority of

roots. For this reason one might expect the reversed direction of the response but at the end of the series when the contacts were returned to the apical 5 millimeters (curve A′) the polarity potential was between 30 and 40 millivolts *positive* and the response continued to cause an increase in potential. In most experiments in which a reversal of polarity of the apex occurred (the potential became negative), the current flow still produced a decrease in positivity (fig. 4, curve B between 100 and 120 minutes).

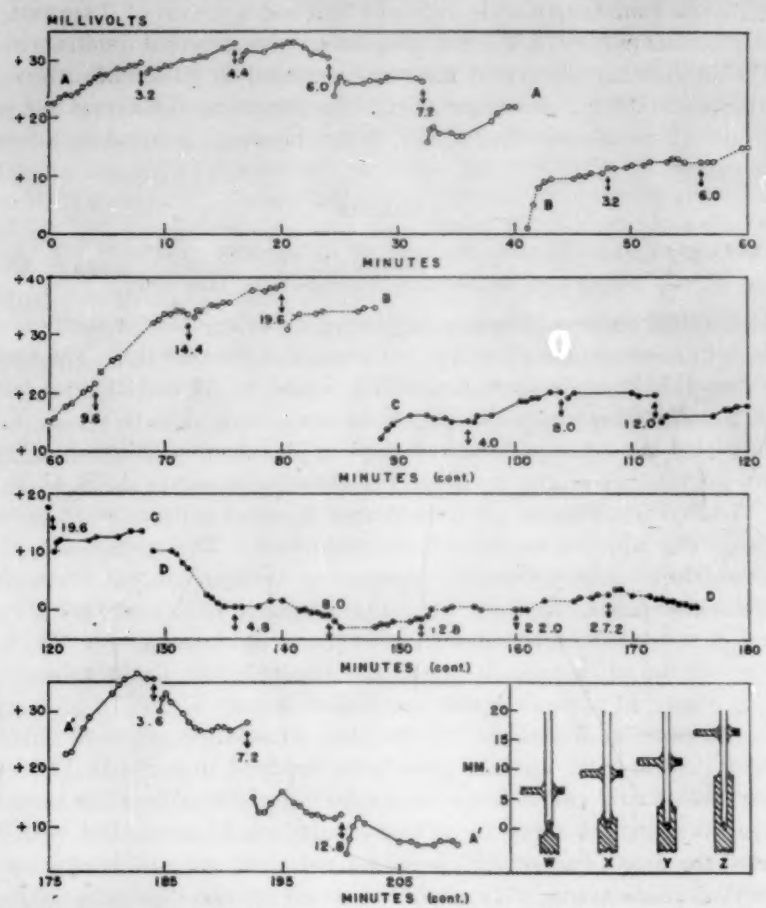

FIG. 1. The response of inherent potential to alternating current. Double-headed arrows indicate AC sent through the root for 30 seconds. The number with each arrow gives the magnitude of the current in r.m.s. (root mean square) microamperes. The positions of the contacts were: Curve A, 0 and 5 mm. (inset W); curve B, 3 and 8 mm. (inset X); curve C, 5 and 10 mm. (inset Y); curve D, 10 and 15 mm. (inset Z); curve A′, 0 and 5 mm. All curves are from the same root.

Both curve B and curve C of figure 2, obtained with the contacts at 3 and 8, and 5 and 10 millimeters above the tip, respectively, insets Y and Z, gave the same type of response as the tip. The strength of current necessary was higher in each case than that required for curve A but the magni-

tude of change was approximately the same. The abnormal behavior of this root must indicate an exceptional condition in the cells since it was the only one of dozens used during the course of the investigation that gave this interesting response. It is hoped that further experiments will yield an explanation of the results.

Since figures 1 and 2 show the first five millimeters of the root tip to be most easily stimulated, it was considered desirable to determine which millimeter segment of the five was primarily responsible for the observed potential change. The current was increased until an appreciable response was obtained and approximately this current was then applied in the same way

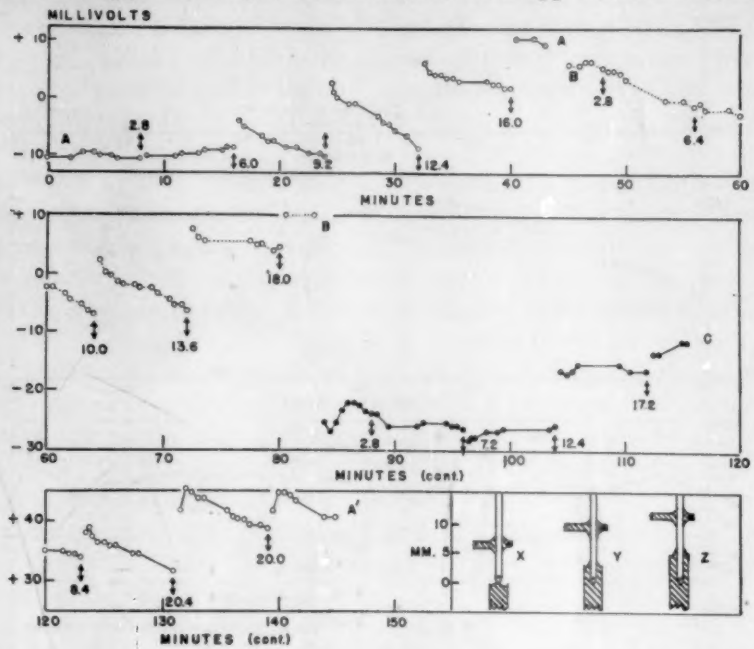

FIG. 2. Same as figure 1. The positions of the contacts were: Curve A, 0.0 and 5 mm. (inset X); curve B, 3 and 8 mm. (inset Y); curve C, 5 and 10 mm. (inset Z); curve A′, 0 and 5 mm. All curves are from the same root.

twice each to the five one-millimeter segments in the first 5 millimeters of the root tip. The contacts were then returned at the end to 0 and 5 millimeters above the tip and the same current applied twice and finally to 1 and 5 millimeters above the tip and current again applied. Figure 3 gives the result typical of the four roots tried.

Curve A is for the entire 5 millimeters and 10.4 microamperes produced a response of 11.5 millivolts. With the contacts at 0 and 1 mm. above the tip, 11.2 microamperes gave a response of 14.5 and 19.5 millivolts (curve B). The slight increase in current resulted from the decreased impedance of the shorter segment of root and the inability to set the potentiometer so as to give a predetermined current. Curve C had the contacts at 1 and 2 mm.

above the tip and 12.0 microamperes did not stimulate the segment. Similarly, curves D, E, and F with the contacts at 2 and 3, 3 and 4, and 4 and 5 mm. above the tip, respectively, gave no response with about 11.5 microamperes. When the contacts were returned to 0 and 5 mm., 10.8 microamperes produced responses of 24.5 and 16.0 millivolts. Finally, with the contacts at 1 and 5 millimeters above the tip no stimulation resulted from

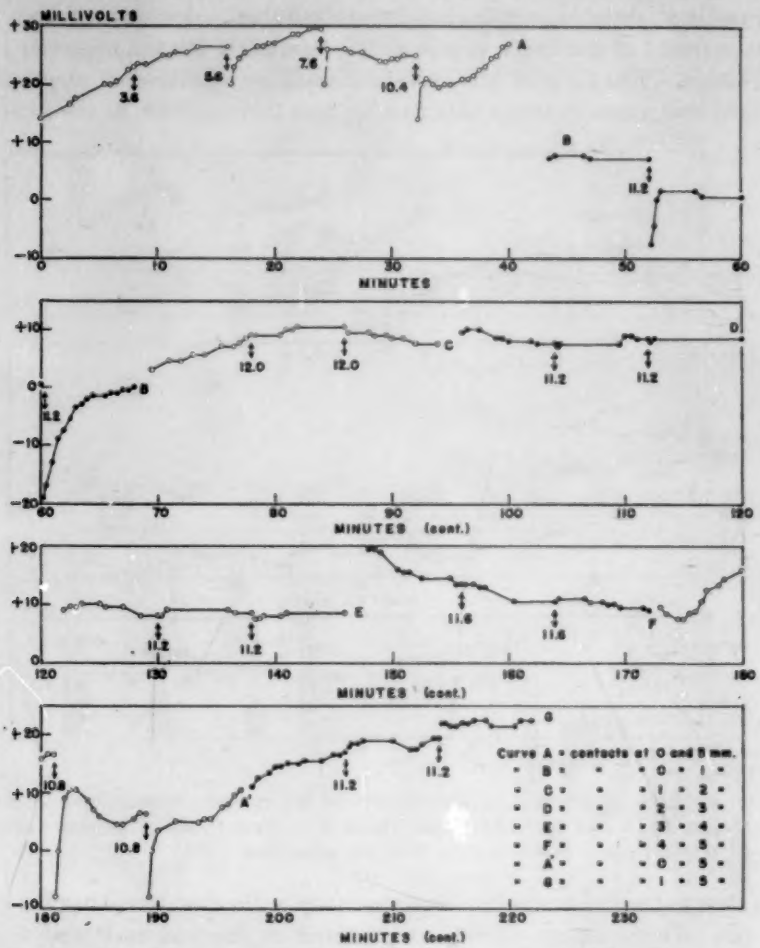

FIG. 3. The effect of segment length and position on the response to alternating current. Double-headed arrows indicate the AC sent through the root for 15 seconds. The number with each arrow gives the magnitude of the current in r.m.s. microamperes. The positions of the contacts are given in the table. All curves are from the same root.

current flow. Hence it is apparent that the first millimeter of the root tip is the part most easily stimulated with alternating current. With that segment removed from the circuit by raising the lower contact, no potential change resulted from the flow of what had been a stimulating current. In this case the results may be due to the greater current density over the first

millimeter of the tip but for more basal segments (fig. 1) the observed differences in response to current flow are not so simply explained.

Thus the flow of AC through a root normally decreases the positivity of the electric potential as measured but in exceptional cases the response to current flow may be oriented in the opposite direction. A response is more readily obtained in apical than in basal segments of the root and with smaller currents.

Effect of alternating current of constant magnitude applied to the root for increasing lengths of time

Beginning with a time of 5 seconds, an alternating current which produced a response was allowed to flow through the root and its effect on potential observed. Eight minutes later the same current was passed through the root for 10 seconds and so on with 5-second increments up to 30 seconds and finally 60 seconds. Curves A and B, figure 4, are for the first 5 mm. of two different root tips and curve C is for 5 and 10 mm. above the tip of the same root as that of curve A. In the latter, 9.6 microamperes for 5 seconds decreased the potential 41.5 millivolts and as the time of current flow progressively increased to 10, 15, 20, and 25 seconds the responses became smaller until finally neither the 30- nor the 60-second flow produced a change. A return to 5 seconds gave a decrease of only 9.5 millivolts (compared to the original 41.5 mv.), followed by no effect for 30 seconds and only 6.0 millivolts for another 5-second application. This change in the response of the root to current flow as the duration of flow increased took place with the magnitude of the potential essentially constant at about 20 millivolts positive after the first application. A similar effect was obtained with the segment of root 5 and 10 millimeters above the tip, curve C, but in this case no response resulted after a 25-second flow of 21.2 microamperes and only a 1.5 millivolt decrease was produced by the final 5-second application as compared to the initial 6.5 mv. change.

A slightly different effect is shown in curve B beginning with an application of 6.0 microamperes for 5 seconds that gave a 30 mv. response which gradually decreased to 7.5 mv. at both 20 and 25 seconds, increased to 15.5 mv. for 30 seconds, and to 12.0 mv. for 60 seconds. Again the return to a 5-second flow gave a smaller response (15.5 mv.) than originally as did the 30-second (5.0 mv.), and final 5-second flow (10.0 mv.). With the same series of current applications repeated using a larger current (10.8 microamperes) it is seen that the magnitude of response regularly decreased up to the 60-second flow, except for the 5.0 mv. greater decrease at 10 seconds than at 5 seconds. Here also the final 5-second flow produced as great a response as the initial one. The decrease in response that generally occurs as the duration of current flow increases is in close agreement with the similar experiments for direct current (3) save that in the latter case the potential change due to polarization is summed with the progressively smaller potential change due to stimulation and the net result may be an increase in potential when current passes up the root for longer time intervals.

The application of current of constant magnitude and duration does not produce a progressively smaller response if the period between applications permits recovery of the potential to approximately the original value (fig.

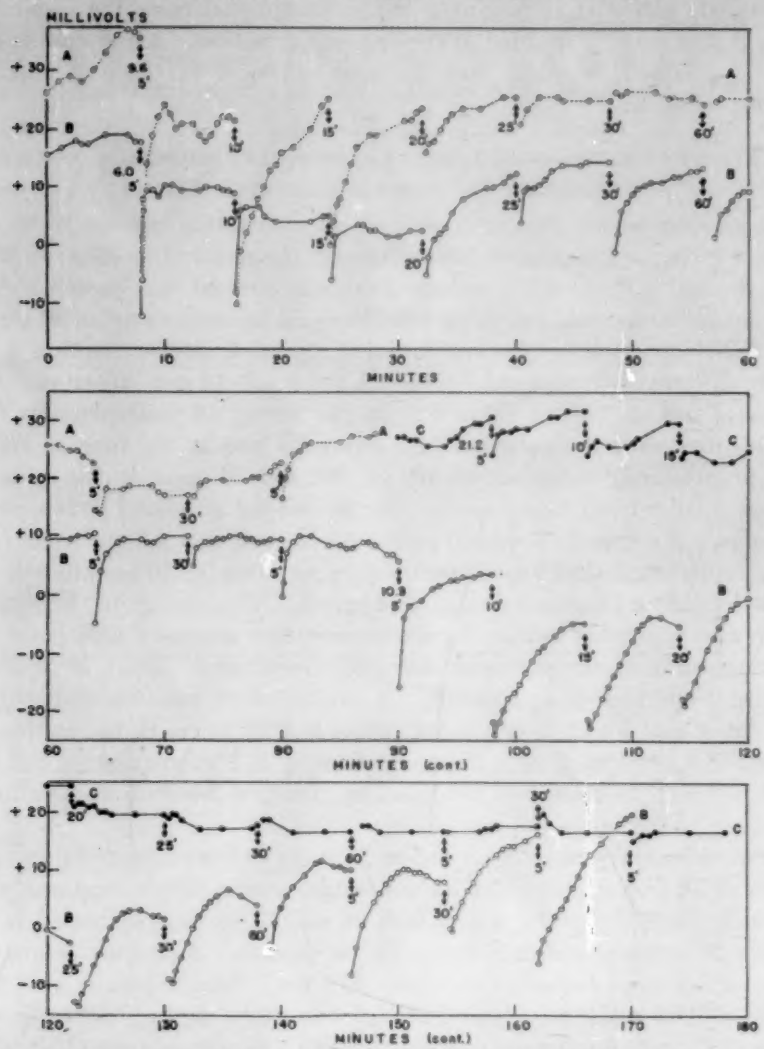

FIG. 4. The response of inherent potential to alternating current of constant magnitude but varying duration. Double-headed arrows indicate the application of AC to the root. The numbers with each arrow give the magnitude of the current in r.m.s. microamperes, and the duration of current flow in seconds ('). When no magnitude is given it was the same as that last indicated. The curves A and C refer to one root, and curve B to another. The positions of the contacts were: Curve A, 0 and 5 mm.; curve C, 5 and 10 mm.; curve B, 0 and 5 mm.

5, first part of curve A). Even so, under such conditions, variations in the magnitude of potential-decrease following current flow may occur. Such

variations, however, are never as orderly as the decrease obtained with longer intervals of application.

Effect of alternating current of constant magnitude applied with shorter time intervals between applications

A stimulating current was applied for 30 seconds to the first 5 mm. of the tip every eight minutes for four times. It was then passed through the root four times for the same length of time but with four minutes between,

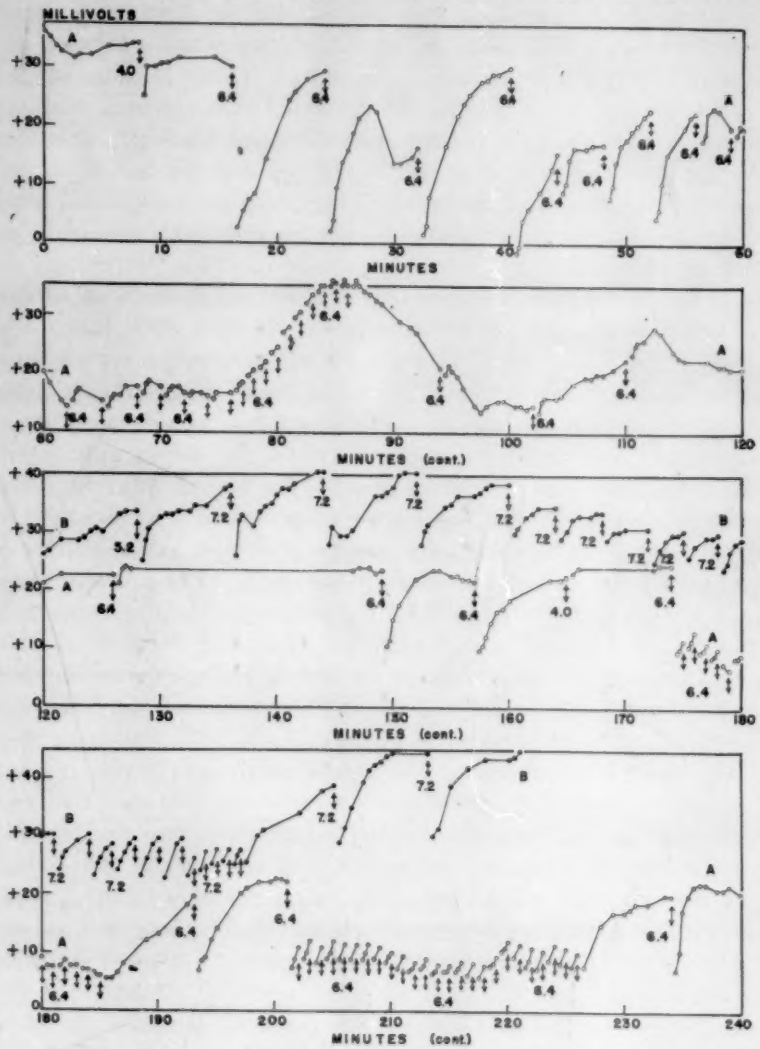

Fig. 5. The effect of decreasing the time between applications of alternating current. Double-headed arrows indicate the application of AC for 30 seconds. The numbers with each arrow give the magnitude of the applied current in r.m.s. microamperes. Curves A and B refer to two different roots. The positions of the contacts were: 0 and 5 mm. for both curves.

then similarly with three, two, and one minutes between applications. Of the four roots so treated two are shown in figure 5. The first part of curve A shows a behavior unlike the other three but the last part of that curve exhibits typical behavior. It should be noted that 6.4 microamperes gave a much larger decrease in potential than 4.0 microamperes and was therefore used for the series in curve A. The four applications of current with eight minutes between, result in three of four responses of about equal magnitude (30 mv.) with the third only half as great. The responses with four minutes between current flow increased in size the first three times and decreased the last. With three and then with two minutes between current applications, virtually no potential change took place. Because one minute intervals between current flow was accompanied by a potential increase, the series was extended until a constant potential was reached. This increase in inherent potential as a result of current flow and the failure of the root to respond at the three and two minute intervals is the exceptional behavior. Eight minutes following this last series the same current was again passed through the root and no response was produced. This "refractory state" persisted for 64 minutes as shown by an occasional application of current during this time and then at 150 minutes on the time scale, the root again responded. The magnitude of this and the following responses was approximately the same as the smallest of the initial four. The root even after the return of irritability was not as easily stimulated as originally since 4.0 microamperes failed to have any effect. Moreover, eleven applications of current one minute apart failed to produce the rise in inherent potential, and two applications eight minutes apart gave normal responses. This was finally followed by twenty-five applications of current at one-minute intervals and all but one caused a decrease in potential. The inherent potential returned to its initial level after this final series and eight minutes later the root was again stimulated by current flow. Thus after recovery of irritability a treatment more drastic than the one initially responsible for the "refractory" condition failed to cause another period of lost irritability.

The root in curve B of figure 5, which starts at 120 minutes on the time scale, required 7.2 microamperes for a good response. It was treated the same as the root in the first part of curve A and responded each time current passed through the root regardless of the length of the recovery period. At the end the original potential level was reached and the size of the response equalled the one at the beginning. The only noticeable effect of applying current with a short recovery period between applications in this case, as well as in the latter part of curve A, was to permit less recovery from the former stimulation and hence less potential decrease. Throughout the experiment in curve B the lower level of the potential immediately following current flow was remarkably constant at about 25 mv. positive. A similar condition is to be seen in curve A over the latter half where the potential was between 5 and 10 mv. positive.

Summarizing, it is found that AC applied several times in rapid succession prevents complete recovery and decreases the observed electric potential

to approximately the same initial level after each current flow. However, this treatment seems capable under some conditions of giving rise to a "refractory state" in the root during which time the applied current produces no response in the root.

Effect of alternating current followed by direct current on the potential of the apical five millimeters of the root

Berry and Hoyt (3) have shown that a root tip stimulated by direct current could not be stimulated again when current of the same strength

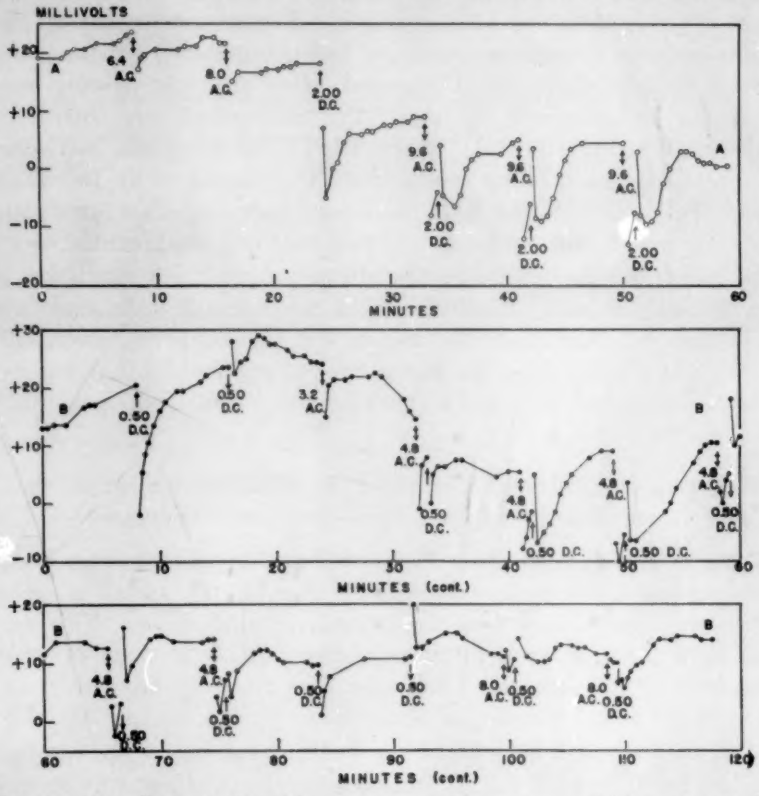

Fig. 6. The effect of alternating current on the response of inherent potential to applied direct current. Double-headed arrows indicate the application of alternating current for 15 seconds, single-headed arrows indicate the application of direct current up or down the root for 15 seconds. The numbers at each arrow give the magnitude of the current (AC or DC) in microamperes. The positions of the contacts were 0 and 5 mm. for all curves. A different root was used for both curves.

was sent through the root within one or two minutes. Instead, the second application of current polarized the root when it opposed the inherent flow of current. In order to see whether a similar effect would result with AC, direct current of stimulating magnitude was sent up the root one minute after the potential was decreased with AC. Typical results with the con-

tacts 0 and 5 mm. above the tip are given from two different roots in figure 6. Current was applied for 15 seconds in all cases. The root that gave curve A could be decidedly stimulated with 2.00 microamperes DC and 9.6 microamperes AC. When the DC was applied one minute after the AC a polarization was produced which was almost perfectly repeated three successive times with eight minutes between the two current applications.

In curve B, 0.50 microampere DC stimulated the root when sent either up or down the root. The latter type of response (an increase in potential after direct current is sent down the root) is not as commonly seen as the former. This response of the root made it possible to determine the effect of both types of DC after AC stimulation. Direct current sent up the root one minute after 4.8 microamperes AC had stimulated it produced another decrease in potential. When repeated after an eight minute recovery period, the DC polarized the root. The same effect was observed after another eight minute period. When DC passed down the root after the AC application, an increase greater than that produced by DC alone was found. This was repeated with the same result. Another application of DC up the root, following the AC, at first increased the potential to a small extent which quickly fell below the initial potential. A repetition of the DC alone, once in each direction, showed the root still to be stimulated by each. Then by increasing the AC to 8.0 microamperes, practically no response was observed and the direct current application that followed increased the potential when sent up the root and decreased the potential when sent down the root.

Effect of alternating current of increasing magnitude on the irritability of the root to direct current

It has been suggested above (sections 2 and 3) that the root is capable of undergoing a decrease in irritability even to the extent of becoming "refractory" under some conditions of current application. This has been found to be the case when sufficiently strong AC is used on the first five millimeters of the root tip. Not only does the root become practically "refractory" to AC stimulation but also to DC stimulation. At the same time, the root can still be polarized with DC. Typical of the results from several different roots is that shown in figure 7. Current was applied for fifteen seconds each time and eight minutes elapsed between applications. Three different magnitudes of DC were first sent up the root and in each case a rather large decrease in potential was produced. Two applications of AC of increasing strength also stimulated the root and another DC (0.50 microampere) again decreased the potential but not as much as originally. By increasing the AC still more no change in potential occurred when 12.0 microamperes was used at 64 minutes on the time scale. This was followed by five applications of 0.50 microampere DC with the first two polarizing and the last three stimulating the root. When 12.0 microamperes AC was again sent through the root it produced a sizable response which became

successively smaller on the next two applications. Beginning at 136 minutes, the AC was increased each time it was applied and each response was smaller until no change resulted with both 40.0 and 47.2 microamperes. The

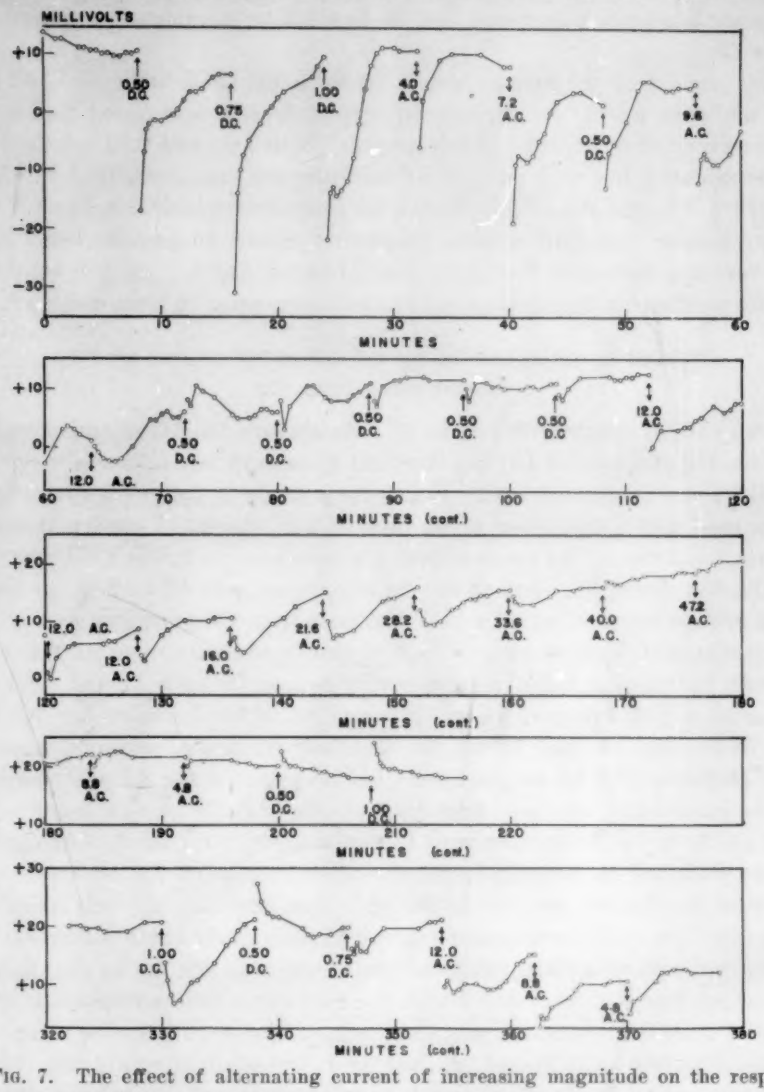

FIG. 7. The effect of alternating current of increasing magnitude on the response of inherent potential to applied direct current. Double-headed arrows indicate the application of alternating current for 15 seconds, single-headed arrows indicate the application of direct current up or down the root for 15 seconds. The numbers at each arrow give the magnitude of the current (AC or DC) in microamperes. The contacts were at 0 and 5 mm.

magnitude of AC which originally gave the largest responses now produced very small potential changes and both 0.50 and 1.00 microamperes DC polarized the root. *The root was now in a "refractory" state and this condition existed when the inherent potential was higher than at any previous period.*

In fact the increase in inherent potential took place during the hour (120 to 180 minutes) when the highest AC was being passed through the root. This increase in potential should be compared with the rise shown in curve A, figure 5, of this paper and that of figure 9 in the paper by BERRY and HOYT (3).

By permitting the root to remain undisturbed for a little over 100 minutes and then testing its response to current flow, it was found that a partial recovery of irritability had occurred. Both 1.00 and 0.75 microampere DC stimulated the root while 0.50 microampere only polarized it. With AC, 12.0, 8.8, and 4.8 microamperes all gave appreciable responses. This rather drastic treatment of roots frequently caused an angular bend in the first few millimeters of the tip by the following day but growth continued and no permanent damage beyond the bending seems to have occurred.

EFFECT OF TEMPERATURE ON THE RESPONSE OF THE ROOT TO ALTERNATING CURRENT

In order to compare the effect of temperature on AC stimulation with that for DC stimulation (3), an identical procedure was followed except for the difference in current used. Reference is made to that work for the technique used and a discussion of its limitations. Essential confirmation was obtained and two of the seven curves are presented in figure 8. Curve A is for the first five millimeters of the tip of one root and curve B is for the 10 to 15 millimeter segment of a different root tip. Current was applied for 30 seconds in all experiments. The first sizable response required 6.4 microamperes for curve A (small responses may be seen for both 3.2 and 4.8 microamperes) and 12.8 microamperes for curve B. After lowering the temperature to between 14° and 15° C. for one hour the apical segment showed a large response with 4.8 microamperes but no response for 3.2 microamperes. On the other hand, the basal segment was only stimulated to a lesser degree with nearly twice as much current as that originally required. As the temperature increased the magnitude of current necessary for an appreciable response also became greater in the apex and exceeded the original value. This condition might well have been due to the greater surface conductivity of the root resulting from condensation of water as the air in the chamber became warmer and more moist with the root temperature remaining lower. Finally, with the return to room temperature approximately the same magnitude of current stimulated the root as in the original condition. In the basal segment, however, the return to room temperature lowered the strength of the current required for stimulation to approximately its former value. However, with all roots the basal segment did not behave in such direct contrast to the apical segment and, as with DC stimulation, the effect of a lower temperature was much more pronounced and definite in the apex than in the base. Thus as shown in curve A, the strength of current for stimulating an apical segment of root is decreased by lowering the temperature; this verifies the results obtained with DC stimulation.

Effect of hydrogen and oxygen on the response of inherent potential to AC stimulation

When oxygen is removed from the environment of a root by passing hydrogen through the chamber, direct current which stimulated the root

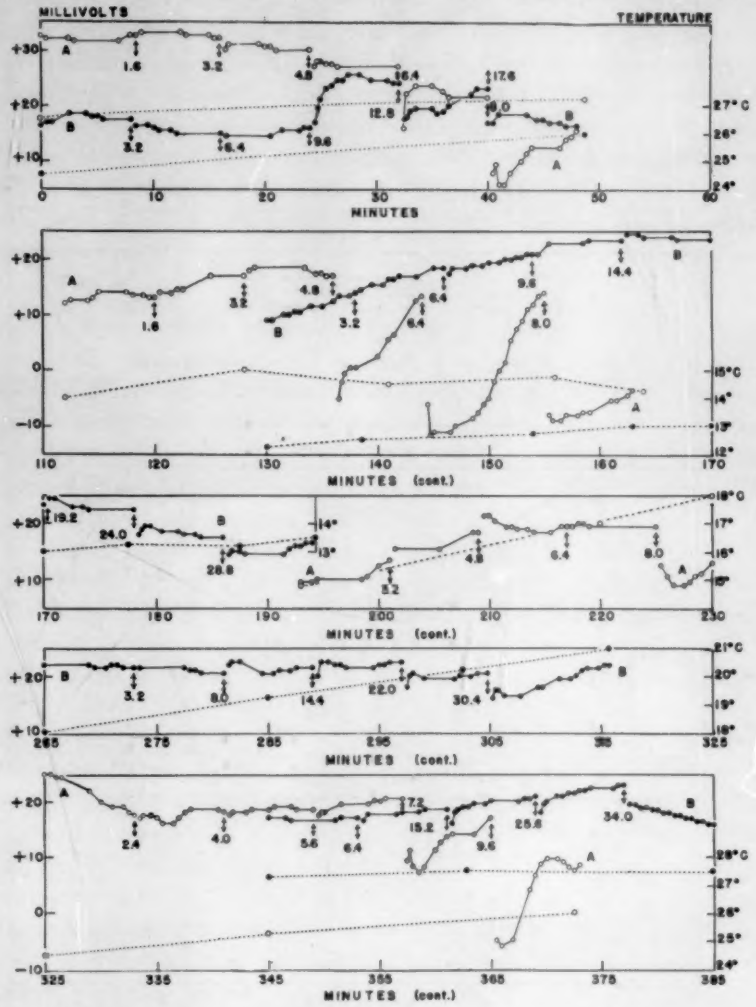

Fig. 8. The effect of temperature on the response of the root to alternating current. Double-headed arrows indicate the application of alternating current for 30 seconds. The numbers beside each arrow give the magnitude of the current in r.m.s. microamperes. The variation of temperature with time is given by the broken curves. Curve A and B refer to two different roots, and the positions of the contacts were: curve A, 0 and 5 mm.; curve B, 10 and 15 mm.

only polarizes it. This effect is reversible by replacing the hydrogen with oxygen (3). It was considered important to observe the change a similar treatment would produce on the alternating current stimulation. The

curve in figure 9 is typical of this action and shows that without oxidative metabolism no stimulation was possible but that irritability returned with a return of oxygen. Moreover, the reversibility of the effect was shown by

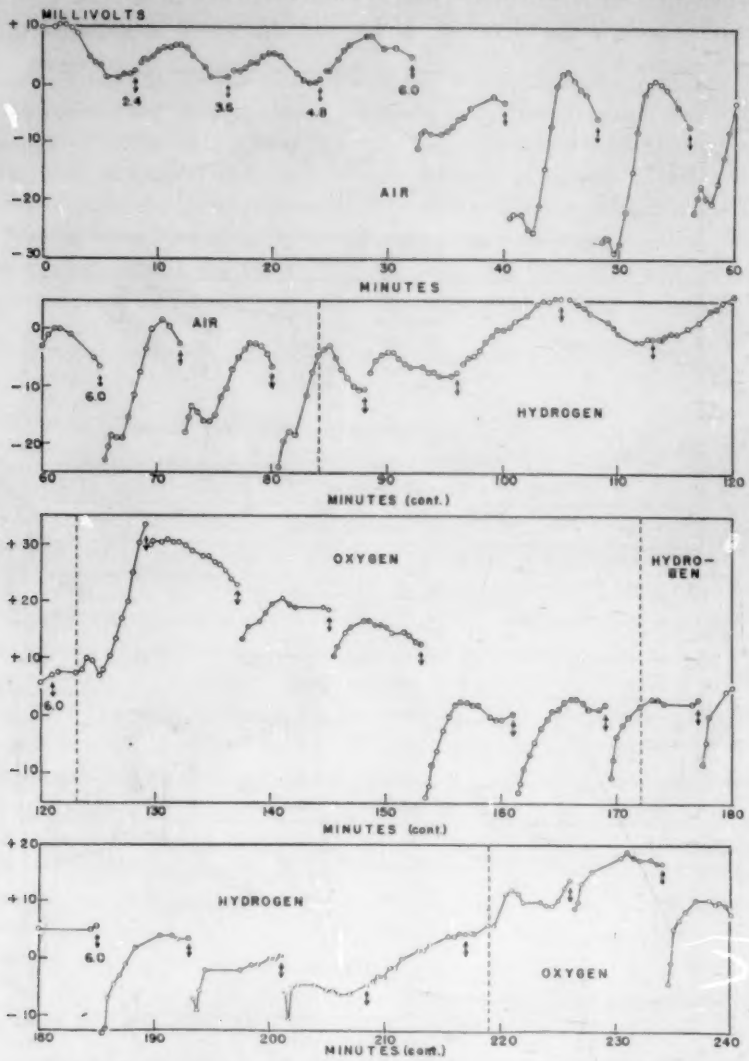

FIG. 9. The effect of hydrogen and oxygen on the response of inherent potential to alternating current. Double-headed arrows indicate the application of alternating current for 30 seconds. The numbers at the arrows give the magnitude of the current in r.m.s. microamperes (when no number is given the current was the same as that last indicated). The dotted vertical lines indicate the time at which the circulation of hydrogen or oxygen was begun. The contacts were at 0 and 5 mm.

repeating the oxygen removal and readmission. The second period in hydrogen required a longer time before the loss of irritability appeared; this might easily be attributed, however, to the fact that hydrogen had to replace

only air the first time and oxygen the second time. A sufficiently low oxygen tension would naturally be slower in appearing in the latter case. This particular curve is of additional interest because at the beginning of the removal of air by hydrogen at 84 minutes the potential was slightly negative and yet was responding to current flow with potential changes of approximately 20 mv. In hydrogen the potential at which no response occurred after applying AC was at first almost the same as before and later was even higher. Thus the absolute magnitude of potential is not directly involved as a cause and effect mechanism. This fact would not have been evident in the other roots of this series since normally there is a fall from a positive PD to essentially zero when hydrogen replaces air. The change in the response of a root to current flow, however, follows a similar pattern in all the roots tested.

Discussion

Alternating current when sent through an onion root could alter the observed electric potential: (1), as a result of rectification by the root comparable to the action of various tissues reported by METZNER (**6**); or (2), by stimulating the root, thereby producing a greater negativity in observed potential similar to the effect sometimes observed with direct current (**3**). Neither of these explanations can fully account for all of the experimental facts as shown by the analyses that follow.

The rectifying element in the root would have to possess the following characteristics: (a) its resistance to upward flow of current should be greater than its resistance to downward flow of current, thereby normally resulting in depolarizing the root, but this behavior should be capable of reversal under some conditions giving rise to results like those shown in figure 2; (b) it must be dependent on oxygen for the maintenance of its rectifying properties (fig. 9); (c) it must be either less efficient or absent in more basal segments of the root compared with the apical segment and especially the first millimeter of the root tip (figs. 1, 2, 3); (d) its effectiveness should decrease with duration of current flow (fig. 4), with larger currents (fig. 7), and at times with increasing frequency of application of current (curve A, fig. 5); and (e) its efficiency must be somewhat greater at lower temperatures especially in apical segments (fig. 8). In some respects it might seem that rectification is produced by the same structures responsible for the oxygen sensitive inherent PD but the disappearance of rectification in curve A, figure 4 between 40 and 60 minutes and in curve A, figure 5 between 90 and 130 minutes without any appreciable alteration of inherent potential makes this doubtful. Moreover, the fraction of total current flowing through the root that is rectified must be small enough not to be detected by the cathode ray oscillograph. Also no stimulation of the root with AC seems to occur even with relatively strong currents but on the basis of DC results (**3**) one might expect this to occur occasionally.

A stimulation of the root with AC can account for all of the observed results except the behavior of curve A, figure 4 and curve A, figure 5 men-

tioned in the paragraph above. It is difficult in the former case to understand how the observed potential can return to its original value during current flow when it is decreased during the first interval of flow. In the second case, the recovery of irritability at constant PD implies that there is no immediate relationship between the structures responsible for inherent potential and those which are thought to be broken down by the stimulating action of current flow (**3**). But with these exceptions all other observations agree in essential detail with the behavior of the roots stimulated with direct current (**3**). There seems to be no doubt but that DC applications may have one of two types of effect on the PD of the root and in some cases the change in potential observed may be an algebraic summation of the two.

The following points of agreement between the response of the root to applied AC and to DC stimulation may be cited: (a) the apical 5-mm. segment of a root is more readily stimulated than more basal segments, and when the latter are stimulated stronger currents are required (fig. 1); (b) stimulation by downward flow of DC (increase in the positivity of observed PD) occurs less commonly than stimulation by upward flow of current, hence the fact that curves like figure 2 appeared with only one root might be expected for this reason; (c) roots stimulated with AC can be again stimulated or polarized with DC of stimulating strength when applied before recovery is complete, the response to DC depending upon the amount of recovery from the AC stimulation (figs. 6, 7); (d) a lower temperature reduces the strength of current required to stimulate the apical segment of a root (fig. 8); and (e) the root cannot be stimulated in an atmosphere of hydrogen but can be stimulated in air (fig. 9).

It is probably true that there is no great choice between the two possible interpretations but it is the feeling of the writers that the latter is preferable because of the close agreement with the DC experiments. Work planned for the near future should make it possible, however, to base that choice on a sounder foundation.

Summary

1. The inherent potential of onion roots may be decreased by the application of alternating currents of sufficient strength. Only one exception was found and in this root the potential became more positive. No change is produced in the root PD unless "threshold" intensities are employed. These responses are more readily obtained in apical than in basal segments. In fact, when a stimulating current of an apical five millimeter segment is applied to each of the one millimeter segments, only the first millimeter of the root is stimulated and no change in PD is observed when it is not in the circuit.

2. As the duration of flow of a stimulating current increases from 5 to 60 or 120 seconds, the magnitude of root response becomes progressively smaller and may disappear. A return to the shorter flow of 5 seconds again

produces a large response, although not as great as initially. If a long recovery period is allowed the magnitude of the response may return to its former value.

3. If the times between applications of current are decreased, recovery of potential may not be complete, but the initial value after flow remains approximately the same. In some roots it is possible by this treatment to induce a "refractory" state, lasting for some time, during which no stimulation is possible. Eventually the root recovers its ability to respond.

4. When direct current of stimulating intensity is applied to a root whose potential has been lowered by alternating current of sufficient magnitude, it will no longer be stimulated but will only be polarized. The same thing will occur if the root has been made "refractory" with large alternating current.

5. The "threshold" for AC stimulation is less at low temperatures than at high temperatures. This effect is reversible and is more pronounced in apical segments than in basal segments.

6. A root which can be stimulated in air will no longer respond after a period in hydrogen, but will again respond when oxygen is admitted. This effect is perfectly reversible.

7. The results are interpreted as being due to the stimulating action of alternating current but the possibility of rectification by the root is discussed.

It is with pleasure that we acknowledge the technical assistance of MISS ATHLEEN JACOBS so faithfully rendered during part of the investigation.

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QUANTITATIVE MEASUREMENT OF THE VELOCITY OF WATER ABSORPTION IN INDIVIDUAL ROOT HAIRS BY A MICROTECHNIQUE

HILDA F. ROSENE

(WITH SIX FIGURES)

POPESCO (**11**) credits the first observations on the absorption of solutes by roots to the work of MARCELLUS MALPIGHI in the seventeenth century. POPESCO writes that, in his work entitled *Anatome Plantarum* which appeared in 1675, MALPIGHI maintained that nutritive substances were absorbed by the roots at the level of the region of the root hairs. Since not all plants bear root hairs, MALPIGHI stated further that the entire epidermal surface functioned in absorption when root hairs were absent.

According to POPESCO, many early investigators following MALPIGHI were not in agreement with these conclusions: GREW in 1682 said that the roots absorbed water and nutritive substances from the earth through their tips, especially through the root cap; HALES in 1727 and DE LA BAISSE in 1733 supported GREW's contention stating in addition that the rôle of root hairs was secondary. Many of the observers in the beginning of the nineteenth century also believed that water and solutes were absorbed through the very tip of the root but in 1865 SACHS contended that the plant absorbed water from the earth by means of the root hairs, that the surface area of absorption was greatly increased by the presence of root hairs, and that volume absorption was proportionally increased. Subsequently, SCHWARZ calculated that the epidermal area in roots of corn was increased six times and in roots of peas twelve times when root hairs developed on these roots in moist air (**10**).

Such outstanding investigators of the early part of the twentieth century as JOST, MOLISCH, and PFEFFER also maintained that the principal rôle of water absorption in hairy roots was played by root hairs but several of their contemporaries disagreed, especially DE LAVISON (**11**) and COUPIN (**11**). In 1926 POPESCO published an account of his extensive experiments carried out to determine the absorbing region of the root. From results based on indirect methods he concluded that the presence or absence of root hairs did not perceptibly influence the absorption of solutes and did not modify the limits of the absorbing region: "La présence ou l'absence de poils radicaux n'influence pas sensiblement le phénomène d'absorption des solutions et ne modifie pas les limites de la région absorbante" (**11**, p. 136).

More recent investigations have been made by HÖHN (**7**) and by SIERP and BREWIG (**15**). HÖHN concluded that it was very probable that root hairs were not able to increase the volume of water absorbed in water cultures and that the presence or absence of root hairs changed nothing in the quantitative conditions of water absorption in the various root zones. He

based his conclusions on duplicate potometric experiments with hairbearing and hairless roots of seedlings (*Zea mays*, *Vicia faba*, and *Tradescantia fluminensis*). SIERP and BREWIG, on the other hand, found that the region of greatest water absorption in *Vicia faba* roots was that section fifteen to eighty millimeters from the apex which ordinarily possesses root hairs; whereas the zone without hairs showed an uptake of an insignificant quantity. Their measurements were not made on the same roots which possessed hairs; observations were made on regions of roots which corresponded to the root hair regions of other roots.

Modern texts in botany and more specifically, in plant physiology, written almost three hundred years after MALPIGHI's publications, are in general agreement that the presence of root hairs increases the absorbing surface of roots to a marked extent and that "nearly all water and salts which enter the plant from the soil do so through root hairs" (**14**) which are in intimate contact with the soil particles. Although investigations dealing with the problem of water absorption in roots have been carried out for centuries, no successful attempt appears to have been made to prove by direct means that the root hair *per se* is an absorbing mechanism. Considering the vast amount of literature on many aspects of the general physiology of root hairs, it is surprising to find an absence of conclusive data on the question of whether or not the root hair does actually function as an absorbing mechanism. The numerous investigators who worked on the problem distinguished between regions with and without root hairs and between hairbearing and hairless roots, but they did not distinguish between absorption through the surfaces of a root hair extension of the epidermal cell and absorption through the surface of an epidermal cell without such an extension. Where root hairs are present, absorption might take place through: (a), the surface of the root hair extension; (b), the epidermal area at the base of the root hair extension; (c), the epidermal cells which have no root hair extension and which are located between the hairbearing cells; (d), the hairless epidermal cells distal or proximal to the hairbearing regions.

The problem of absorption of water and solutes by root hairs is one which involves a *single* hair; in a fundamental sense, therefore, it is a problem in cell dynamics since the epidermal cell with its root hair extension is a single cell. The superficial position of the hair cells on the surface of the root, their relatively rapid development and growth, the absence of chlorophyll, and the fact that they are uninucleate make them very favorable material for numerous studies in cell dynamics.

No doubt the chief reason that no conclusive investigation has been made to determine the rôle of the root hair as an absorbing mechanism results from the difficulties of the technique involved in a realm of such small dimensions. As far as the author knows, the present study is the first to furnish quantitative data on the question of whether or not root hairs function in the absorption of water. The technique is a micromodification of that previously used by the author in studies on water absorption in the

onion root (**12**). This micromodification permits observations to be made on a single root hair.

Micropotometers were constructed from glass capillaries and fastened in a horizontal position to a vertical support. Since the hairbearing epidermal cell elongates perpendicularly to the longitudinal axis of the root, the hair extension was readily inserted into a single micropotometer. Accurate measurements of length were possible because the horizon of the root at the base of each hair and the terminus at the apex were definite. Determination of the surface area exposed to the solution in a micropotometer was simplified by the fact that the tubular hair extension is uniform in diameter and ends in a blunt dome-shaped apex. The data reported in this investigation were obtained from the root hairs of radish seedlings (*Raphanus sativus*).

Apparatus

Plates of thin glass, free from aberration, were fastened together with de Khotinsky cement to form the rectangular chamber N shown in figure 1. It is 5 cm. square and 11.5 cm. high. It fits into a depression, A, cut into the base, B, made of transite which had been immersed in hot paraffin to make it water tight. The base, B, which is supported by the rod, S, has four perforations each fitted with a guard made from glass tubing. Through these guards pass, respectively, a thermometer holder, C, a movable glass rod "window wiper," D, a movable root holder shaft, I, and a movable root "adjustor," F. The window wiper consists of a glass rod with a double bend; a piece of rubber tubing placed over the second bend serves to remove moisture from the glass wall facing the horizontal microscope.

The seedling holder consists of a small saddle, E, attached to a small watch wheel in the frame, H, above the saddle. The saddle with the attached seedling is moved on its axis through the action of two watch wheels which are operated by moving the milled head, G, of a rod attached to one of them; this rod moves in the shaft, I, which has a collar, J, to support a clamp; by this means the root may be rotated on its axis. The wheels are thoroughly greased with vaseline and covered by a small rectangular box cut from a piece of lucite rod. The glass root adjustor consists of a section of rod with a double bend at the top end, the tip of which was drawn out to a delicate projection with a blunt curved terminus. The root adjustor was used to place the seedling in a suitable position in the seedling holder.

The micropotometer support, K, is a glass rod pulled out to a very small diameter at the end on which two micropotometers, M, were attached with de Khotinsky cement. It is inserted through the glass guard, L, at the top of the chamber. This glass guard forms a rim 15 mm. in height attached to the edge of a hole 25 mm. in diameter cut through the chamber top.

The glass guards are necessary to permit freedom of movement with horizontal or vertical displacement of the rods which pass through the various openings. In order to provide moisture- and air-tight seals with suffi-

cient movement at the perforations through which the rods pass, latex rubber finger cots are used.

The pair of micropotometers, M, consists of glass capillaries made from carefully selected Pyrex tubing. Short lengths of tubing were pulled into fine capillaries of uniform bore and sections were cut from the capillaries to make the micropotometers. One end of each section was cautiously fired to constrict the inside diameter. This constricted end will be referred to as the proximal opening and the other as the distal end. This constriction was

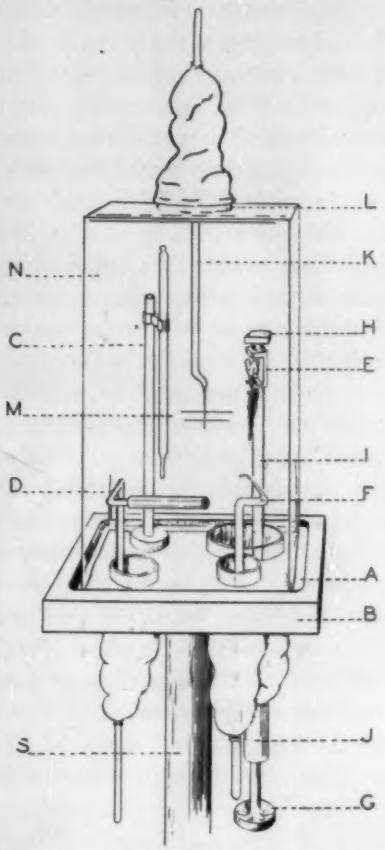

FIG. 1. Experimental chamber. See text for description.

necessary in order to hold the meniscus at the proximal end of the micropotometer capillary. Micropotometers of different inside diameters were used in different experiments but each pair was made from adjacent sections of a pulled capillary in order to match them. Dimensions of a typical micropotometer into which a single hair is inserted are: length, 10 mm.; inside diameter, 58.9 microns; diameter of opening at constricted end, 34.2 microns. The micropotometer capillaries were too fragile to be handled by forceps. Each was handled by placing a minute drop of water on it and taking advantage of surface tension forces.

One micropotometer of each pair served as a control. The pair of micropotometers were cemented to the potometer support in such a way that the micropotometer used to test absorption extended beyond the control and was, therefore, nearer the hairs. With this arrangement it was possible to insert a single root hair into one micropotometer without permitting other hairs to come into contact with the control capillary.

The glass rod, K, supporting the micropotometers and the collar, J, of the root holder support are each clamped to a micromanipulator not shown in the figure. An individual root hair is inserted into the proximal end of the micropotometer by adjustments made with the micromanipulators. All adjustments of the micromanipulators are made with the aid of a horizontal microscope fitted with a Filar micrometer. By this means very delicate adjustments of the positions of either the root holder or micropotometers can be made without mechanical injury to the root hair.

The apparatus used, including the horizontal microscope, was placed within an auxiliary chamber with a wide opening in front covered by a drop curtain of black sateen. This afforded an opportunity to permit development and growth of hairs on a seedling within the experimental chamber in the dark while other work necessitating light could be carried on in the laboratory at the same time.

An indirect source of light is produced by a beam from a light placed at a distance of four feet from the wall of the auxiliary chamber. The beam passes through a flat flask containing a solution of copper sulphate, and penetrates the auxiliary chamber through a small hole. The beam is reflected from a concave mirror which was clamped to a micromanipulator. This provided a concentrated light which could be accurately placed on any region of the micropotometers, hairs, or the seedling. A fluorescent lamp placed within the auxiliary chamber provided a direct source of light. No precaution was taken to use a ruby light since JEFFS (**8**) found, using a wide range of candle powers for various periods of time, that the root hairs of *Raphanus sativus* showed no light-growth reaction. In most instances, in the present work, the fluorescent lamp was used as the light source. It was usually turned on only when making measurements or setting up an experiment.

Procedure

The seeds were soaked for 24 hours and then germinated on wet filter paper in Petri dishes placed in the dark. The experiments were conducted in a fair-sized basement room with thick walls. It was possible to maintain a practically constant temperature when the room was not used for other experimental work involving the use of lights, etc.

The experimental chamber was partly lined with strips of filter paper; the depression (A, fig. 1) in the transite base and the finger cots attached to the guards in the base were filled with distilled water. In this way the floor of the experimental chamber was flooded with water, and the filter

paper strips were wet since they extended into the water when the chamber was placed in position.

Seedlings were placed in the experimental chamber either a few hours preceding the taking of measurements or the night before. In either case, data were obtained from hairs which developed in the moist atmosphere within the experimental chamber. The general procedure is as follows: the micropotometer support and the attached finger cot are removed from the top of the chamber and fastened in a clamp on a nearby stand. A rubber stopper is fitted temporarily into the guard on top of the chamber. With the experimental chamber in the left hand, a seedling which has a short root is gently removed with forceps from a Petri dish and placed in the root holder where it is secured with a small amount of petrolatum. The chamber is quickly replaced and the glass root adjustor is manipulated from the outside to make necessary adjustments of the seedling position; it is held in an upright position with respect to gravity. The micropotometers are filled and the supporting glass rod clamped to a micromanipulator by means of which it is lowered into the chamber having removed the rubber stopper from its top. The preparation is left undisturbed until readings are to be made. The micropotometers are then moved into a position in close proximity to the hair selected for observation. With the aid of the microscope the hair is threaded into the proximal opening of the upper micropotometer by (a) moving the micropotometer over the root hair or by (b) moving the root holder and thus the hair into the micropotometer which in this case is held in a fixed position. This was possible without permitting the hair to come in contact with the glass surface and thus producing mechanical injury. Difficulty was experienced when the hairs were in close proximity, especially if the micropotometers touched adjacent hairs; root hairs are "sticky" and will adhere firmly to the outside surface of the capillaries.

A special technique had to be developed to clean and refill the fragile micropotometers. Solutions were drawn into the capillaries by placing the proximal end in contact with the liquid. Solutions were forced out of the micropotometer capillaries by applying a fine jet of air or water at the distal end. Much care was exercised in cleaning and refilling the flexible micropotometers immediately following an experiment.

Change in length of the water column within each micropotometer was determined by making consecutive measurements of the movement of the distal menisci with the Filar micrometer. A number of experiments were carried out to determine whether or not movement of the water menisci in the micropotometers occurred in the absence of (a) any seedling in the experimental chamber and (b) in the absence of a root hair in the upper micropotometer when a seedling was growing within the experimental chamber. As will be shown later, movement of the distal menisci in the micropotometers usually occurred in the absence of root hairs or of any seedling within the chamber. Such movement indicated that under the given conditions either evaporation occurred, or temperature changes produced variations in the length of the water column in the micropotometers.

Since no constant temperature, constant humidity room was available, it was impossible to completely control these factors. Although movement of the distal menisci in both micropotometers occurred, the rate of movement in the upper capillary with all or part of the root hair immersed was significantly greater than the rate of the corresponding moving meniscus of the control capillary. The factors responsible for evaporation were unknown. A possible source of evaporation from the chamber itself was the upper latex finger cot around the glass guard, L, of figure 1. Inverting it and filling it with water, however, made an effective seal but movement of the distal menisci in both micropotometers continued. Various small inequalities in vapor tension due to localized unstable conditions on different small surfaces might be responsible for evaporation. It may be that evaporation occurred from the surface of the meniscus at the constricted proximal opening as a result of a localized high vapor tension over its small curved surface as compared to a relatively lower vapor tension over the plane surface of the larger body of water flooding the chamber floor. According to ADAM (**1**) "the vapour pressure over a convex surface is greater than that over a plane; and over a concave surface it is less." He cites data obtained by THOMÄ (**16**) who showed that vapor pressure is greater with a smaller surface. The area of the proximal opening of the upper micropotometer, into which the hair was inserted, was greater than that of the lower micropotometer, which served as a control, by the cross-sectional area of a typical root hair. In this way the areas of the water-vapor interfaces of the proximal openings were comparable although their shapes were different.

In order to determine volume absorption during unit time by a given region of an individual hair cell, the data obtained from volume displacement indicated by movement of the distal meniscus in the root hair micropotometer during each interval were corrected for evaporation by subtracting data obtained from corresponding measurements of any movement of the control meniscus. Calculations of the velocity of water absorption were based upon the calculated volume absorption during unit time and measurements of the length and diameter of the immersed region. Although the apex of each hair was dome-shaped, no corrections were made for the variation in diameter at the extreme tip; the slight variation in area due to curvature was considered negligible in the present experiments.

The technique was subject to errors inherent in microscopic measurements. Errors due to readings were, of course, largest when the rate of movement of the distal menisci was slow since it was difficult to determine precisely a linear displacement of less than 2 microns. Percentage error due to readings, therefore, would depend upon the total linear displacement during any one interval; such displacement would be greater with capillaries of smaller diameter, other things being equal. An example of a relatively large difference in linear displacement during a ten-minute interval is 226 microns in the root hair micropotometer capillary and 16 microns in the control capillary. Similarly, errors in the determination of surface areas

would be proportionately greater in root hairs of comparatively small diameters.

An analysis of the tap water used in the experiments showed the following in p.p.m.:

Total dissolved solids 143

Ca 13.0	CO_3 12	Cl 30.0
Mg 4.5	HCO_3 37	F 0.2
Na 23.0	SO_4 27	NO_3 0.6

Measurements of hydrogen ion concentration showed a pH of 8.2 at 25° C. The osmotic pressure calculated from the freezing point lowering was 0.122 atm. at 25° C.

Results

The diagram below the curves in figure 2 shows a root hair inserted through the constricted proximal opening (p) of a capillary micropotometer, the distal end (d) of which is also illustrated. The dotted line (m) indicates the position of the distal water meniscus at the beginning of an experiment and the arrow the direction of its linear displacement during absorption of water through the surface of the root hair extending into the micropotometer. "Volume movement" or "volume displacement" of the distal meniscus in the control capillary and in the micropotometer containing the root hair, during unit time, was determined by multiplying the linear displacement of the distal meniscus in each by its corresponding cross-sectional area, neglecting curvature of the meniscus. Curve 1, figure 2, shows the average "volume movement" (cu. mm./min.) indicated by the distal meniscus in a capillary micropotometer containing a root hair almost totally immersed during six consecutive ten-minute intervals; curve 2 shows the "volume movement" in the corresponding control capillary. The difference in magnitude of the two curves indicates that a relatively large volume of water was steadily absorbed by the hairbearing epidermal cell through its immersed surface which was 0.0478 square millimeters. This difference in magnitude which gives the calculated volume of water absorbed in unit interval of time during each ten-minute interval is plotted in curve 3 (ordinate on left). Curve 2 shows that a small non-uniform rate of "evaporation" was manifested by the control; the deviations, however, fall within the range which might be expected due to limitations in taking measurements. But the deviations from a constant volume absorption during each ten-minute interval as indicated by curve 3 (fig. 2) are greater than those which might be expected from experimental error; they point to a drift in the average velocity of absorption with time. These variations in the volume of water absorbed by the hair cell and "evaporation" from the control with time were evidently independent of observed temperature change. Curve 4, figure 2, was plotted from calculations obtained by dividing the calculated volume of water absorbed during unit time through the surface of the root hair exposed to the solution by the area of this sur-

face determined from measurements of the length and diameter of the immersed region. It therefore shows the average velocity of absorption of water through the immersed region of the hair cell during each ten-minute interval.

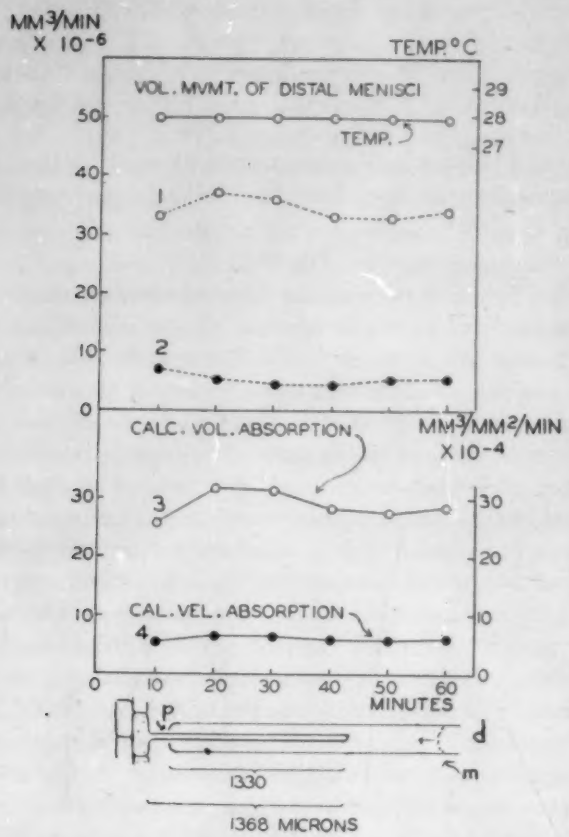

FIG. 2. Water absorption by a root hair almost totally immersed. Data obtained from a seedling which had been placed in the experimental chamber the preceding evening, the hair under observation having appeared during the night, probably during the early morning hours. The seedling was selected from a Petri dish into which seeds had been placed four days earlier. The diagram below the curves shows the length of root hair immersed and the total length of the hair; p indicates the proximal and d the distal end of the micropotometer with the middle section not showing; m indicates the distal meniscus of the solution. Curves 1 and 2 show the average volume displacement indicated by the distal menisci in the root hair micropotometer and the control, respectively, during unit time (ordinate on left). Curve 3 shows the calculated volume of water absorbed by the hair in unit time (ordinate on left) and curve 4 the calculated velocity of absorption through unit surface (ordinate on right). Temperature readings were made at ten-minute intervals and the average of two successive readings plotted as the temperature during the intervals.

It is important to note that in figure 2 and in all subsequent figures the ordinate for the velocity of absorption is on the right and that its scale is

different from that on the left. This was done in order to plot all the curves from a single experiment on one figure. The scale selected was a matter of convenience; the fact that curve 4 is nearer the base line than the other curves has no other significance.

The curves of figure 3 show typical results obtained during experiments of several hours duration. The seedling was placed in the experimental chamber 30 hours after the seeds had been put into a Petri dish to germinate. There were no hairs on the short root when the seedling was placed in the

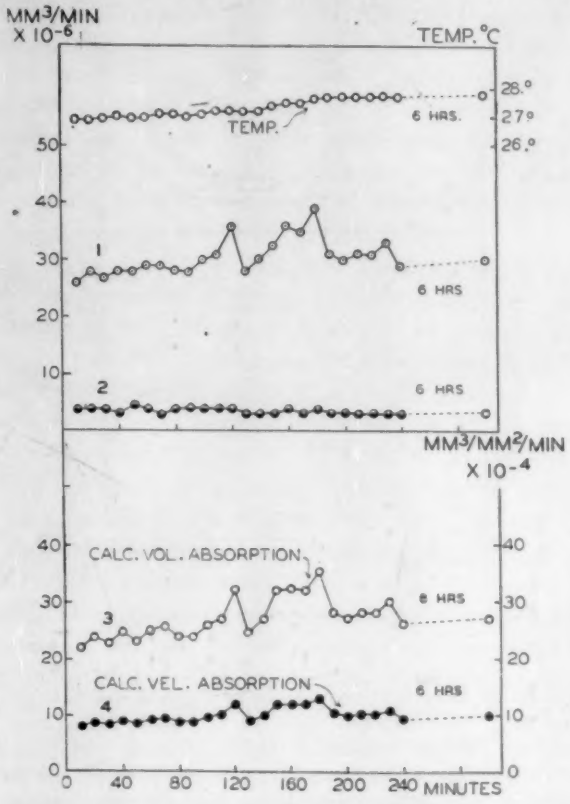

FIG. 3. Results obtained during a relatively long period of immersion. Total length of root hair, 798 μ; length of immersed region, 570 μ; area of surface exposed to water, 0.027 square millimeters. See legend of figure 2 for explanation of the curves. Observations were made at ten-minute intervals for four hours and finally at the end of a 6-hour interval.

chamber the night preceding the experiment. A young hair was selected for observation the next morning. It did not grow during its relatively long period of immersion but subsequent growth occurred in the moist chamber air during the following night. The average rate of evaporation from the control capillary was comparatively low during all ten-minute intervals throughout the first four hours of observation (*cf.* curve 2, fig. 3, with curve

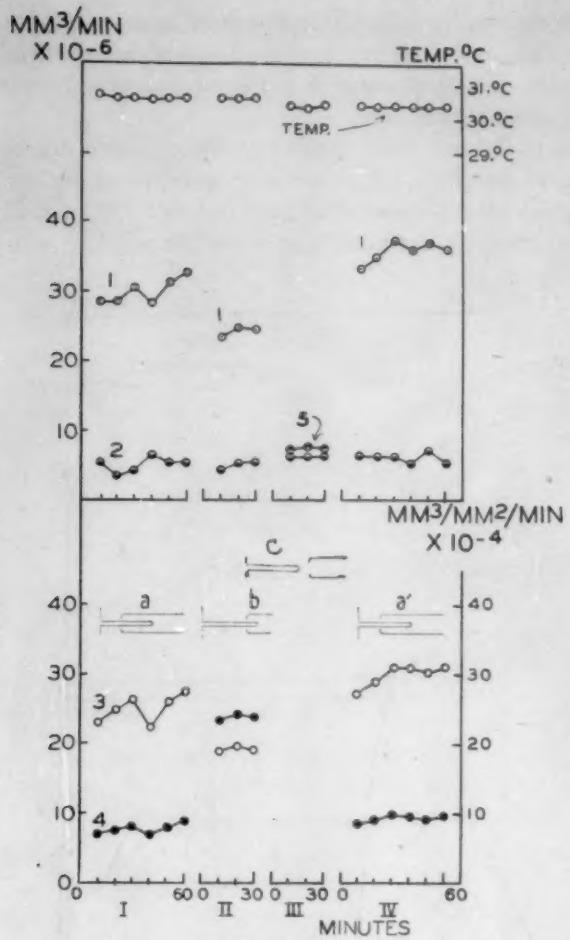

FIG. 4. Absorption when different lengths of a single root hair are exposed during different time periods. The diagrams above curve 3 show the relative lengths of root hair immersed during different consecutive time periods which are numbered in Roman numerals below the base line. The breaks in each curve indicate the time during which the position of the hair in the micropotometer was changed. The different parts of curve 1 are indicated by circles with a dot in the center; curve 2, by circles with lower half black; curve 3, by white circles; curve 4 by black circles. See legend for figure 2 for further explanation of curves 1, 2, 3, and 4. Curve 5 shows volume displacement indicated by the distal meniscus of the root hair micropotometer during unit time after the root hair was withdrawn; in this instance both capillary micropotometers served as controls.

= volume displacement in root hair cap.
= volume displacement in control.
= calculated volume absorption.
= calculated velocity absorption.

2, fig. 2). The average rate of evaporation during the subsequent 6-hour interval was also low. No marked fluctuations in the rate of evaporation were observed. On the other hand, curve 1 shows not only an overall drift of volume displacement indicated by the distal meniscus in the root hair micropotometer but also pronounced deviations from interval to interval, especially after the second hour. The oscillations in curves 1, 3, and 4 are outside the range expected from experimental error; those of curve 2, within this range. Temperature variations also occurred; the temperature increased from an initial value of 26.9° to 28.75° C. at the end of 190 minutes; it then remained constant. The slopes of curves 3 and 4 are, in general, similar to the slope of the temperature curve, indicating a possible temperature effect on the absorbing mechanism of the root hair; the slope of curve 2 indicates a comparative absence of a temperature effect on the length of the water column in the control micropotometer and the rate of evaporation. Curve 4 shows that a fairly high average velocity of absorption of water was maintained throughout the ten hours of immersion.

The curves in figure 4 were obtained from a root hair on a seedling placed in the chamber in the morning. Within a half hour of transference new root hairs appeared. Six hours later one of these hairs was selected to determine the velocity of water absorption through different surface regions along its longitudinal axis. The length of the hair was 1140 microns. Four different time periods are indicated by Roman numerals on the base line in figure 4. The initial temperature was 30.6° C.; it dropped to 30.2° during the third period, then rose to 30.4° in the fourth period. During the first period, which was 60 minutes in duration, observations were made when the distal region immersed was 684 microns in length and the area 0.0324 square millimeters as illustrated in diagram a, figure 4. The root hair was then partially withdrawn from the micropotometer tube until the length exposed was 171 microns and the area 0.0081 square millimeters (diagram b, fig. 4). Observations were again made during a second period which was only thirty minutes in duration. During a third period of thirty minutes duration, the root hair was completely withdrawn as indicated in diagram c in order to determine whether or not the distal meniscus in the root hair micropotometer would continue to move in the absence of the root hair. Finally, preceding a fourth period, the root was again moved (diagram a′) to immerse the same hair region which was immersed during the first time period. Each curve in figure 4 shows breaks; each break indicates the time during which the position of the root hair was changed; the various parts of each curve have the same type of circles and represent data obtained during the corresponding time periods indicated on the base line.

As in the preceding experiments, there was a relatively large difference between the volume displacement indicated by the distal menisci in the control (curve 2) and the root hair (curve 1) micropotometers when the root hair was exposed to water during intervals I, II, and III. When, how-

ever, the root hair was withdrawn (curve 5) the difference was slight. A comparison of the different parts of curve 3 shows that during period II when the hair was in position b and the area exposed was 0.0081 square millimeters, the calculated volume of water absorbed during unit time was less than that absorbed during unit time in periods I and IV when the root hair was in position a or a′ and the area exposed was 0.0324 square millimeters. Although the area exposed, during periods I and IV, was four times greater than that exposed during period II, no such marked difference was observed in volume intake during unit time. This may be partially accounted for by examining curve 4, figure 4, which shows the average velocity of absorption of the surface exposed during the different intervals.

A comparison of the different parts of curve 4, figure 4, shows that *when the length of root hair in the micropotometer was reduced to one-fourth* (from 648 microns in position a, period I, to 171 microns in position b, period II), *the average rate of water intake through unit surface area was tripled.* When, during period IV, the length was again increased to 648 microns (in position a′, period IV), the average velocity of absorption manifested by each unit of surface decreased to its initial value. This indicates that the average velocity of absorption per unit area at the tip of the root hair is much greater than that per unit area in regions relatively more proximal under the particular conditions of the experiment. It does not, however, show whether or not the same velocity obtained at the tip when it was in position a or a′ as when it was in position b. Although the temperature was constant during most of the first and all of the second period, variations appeared in the velocity of intake and the volume absorbed during unit time.

The curves of figures 5 and 6 were also obtained from individual hairs by varying the region observed during different time periods. Observations were made on water absorption by three different root hairs (A, B, C), each on a different root. The three experiments were run on different days; in each the seedling was placed in the chamber the preceding night. Root hair A was 874 microns long; the region inserted into the micropotometer during time periods I and III was 722 microns in length and 0.019 square millimeters in area (diagrams a and a′); during period II the distal half of this area (diagram b) was exposed to water. Root hair B was practically twice as long (1710 microns) as root hair A; half of its length (immersed area: 0.031 sq. mm.) was inserted during periods I and III (diagrams a and a′); one-fourth of its length (immersed area: 0.0155 sq. mm.) during period II (diagram b). An inspection of curves 3 and 4 for each root hair shows that although the calculated volumes of water absorbed per unit interval of time (curve 3) were reduced during period II when the area of absorption was reduced by one-half, the average velocity of absorption (curve 4) was greater than that observed during periods I and III. During period II, almost one-half of the total area of root hair A was exposed to water and one-fourth of the total area of root hair B. It is interesting that in root

hair B where a proportionately smaller region of the distal end of the hair was exposed, the volume decrease in absorption per unit time was less, and the increase in velocity per unit area was greater, than that observed in root hair A; although in period II of each experiment the area exposed had been reduced by one-half. The significance of this fact is more apparent when the variations in the curves of root hair C (fig. 6) are compared.

Root hair C (fig. 6) was 1387 microns in length; the regions immersed in the micropotometer during periods I, II, and III were respectively 760, 380, and 190 microns in length and 0.0224, 0.0112, and 0.0056 square millimeters

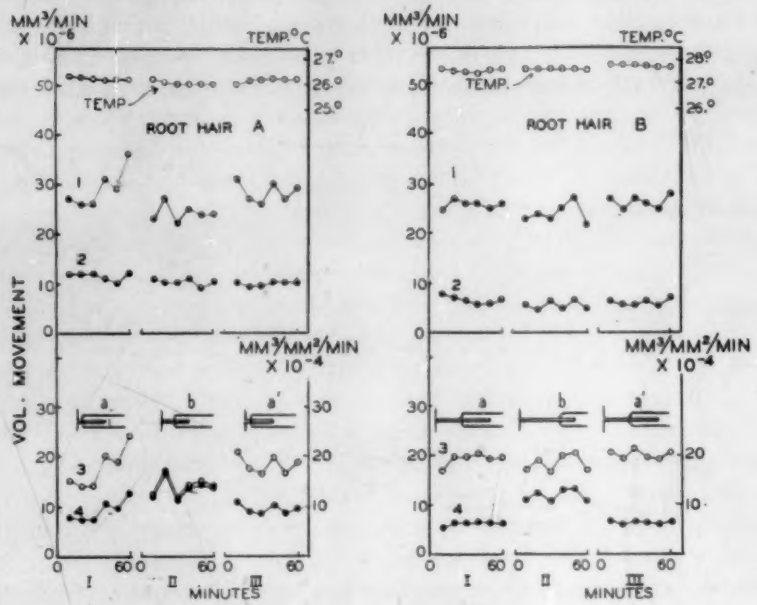

FIG. 5. Absorption by different regions of different root hairs. Curves for two different root hairs (A, B), each on a different seedling, are given. See legends of figures 2 and 4 for explanation of curves.

in area in the respective positions indicated by diagrams a, b, and c. The root hair was then completely withdrawn as indicated in diagram d, period IV, and observations were made on both micropotometers as controls (curves 2 and 5, period IV). Subsequently, observations were made during periods V, VI, and VII when a reverse procedure was used, increasing the area of exposure at stated intervals. The curves obtained during periods III and V are, therefore, comparable as are the curves for periods II and VI, and I and VII—since the same regions were exposed although at different times. The average total volume of water absorbed (curve 3) during unit time is greatest, and the average velocity of absorption (curve 4) is least, during periods I and VII when the areas of exposure were greatest. With diminishing area, a decrease in the average volume of absorption per unit

time and increase in the average velocity of absorption appeared; the reverse was true with increasing area.

Although diminution of area occurred within the micropotometer when the root hair was partially withdrawn, the actual absorbing area might have been greater than the observed diminished area due to a possible residual film of water along the sides of the hair when it was partially withdrawn. If so, the observed increase in velocity was only an apparent increase. Attention, however, is called to the fact that in the experiments from which the curves in figure 6, root hair C, and figure 4 were obtained, the hair was completely withdrawn for over an hour and then reinserted by increasing the area at specified intervals. In further experiments, not reported, similar results were obtained. In the reverse procedure, opposite results would be expected if the observed area were less than the actual area when the hair

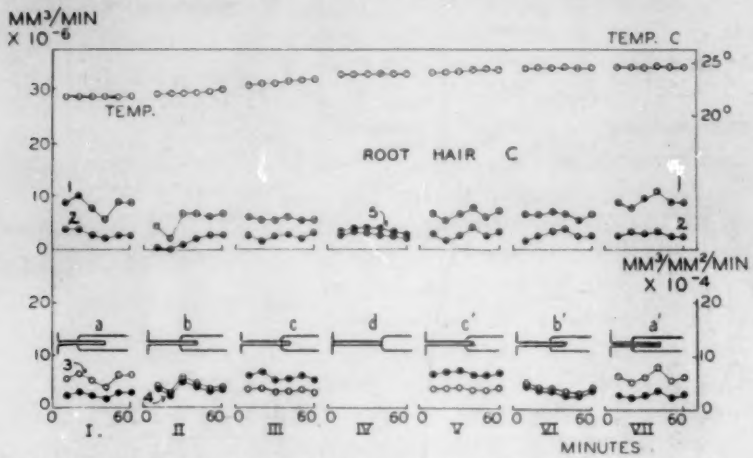

FIG. 6. Absorption by different regions of an individual root hair. See legends of figures 2 and 4 for explanation of curves.

was withdrawn. Hence, the data obtained in the experiments during which the area of immersion was varied in different periods show that when the area of exposure was confined to the relatively distal end of the hair, the average velocity of absorption per unit area was actually greater than that manifested when the area of exposure included not only the distal end but proximal regions also. This indicates either that the average velocity of water entry in the region at the tip was markedly different when the area of exposure was varied and that this difference was due to changes produced by varying the area; or that varying the area did not produce the observed increase in average velocity of absorption, and that this increase indicates a distinct polarity in the distribution of velocity of absorption along the longitudinal axis of the individual hair cell, higher velocities occurring in relatively more distal regions. A final test to determine whether or not such a polarity exists in the type of hairs studied would be simultaneous measurement of water absorption in different regions of an individual hair

employing a technique similar to that used by the author in work on the onion root (**13**). It might also be that a compensating mechanism and a distinct functional polarity are both present.

Discussion

For the first time, after many decades of conflicting opinion and speculation beginning with the early microscopists, quantitative data have been obtained in support of the many writers who have maintained without direct experimental evidence that root hairs function in the process of water absorption. It has been claimed by many workers that root hairs play a primary rôle in absorption of water from the soil. The increase in surface area due to the presence of root hairs has been determined by numerous investigators. But the question as to what extent root hairs will facilitate absorption by increasing the surface exposed to the external solution is one which cannot be answered by mere determination of the surface area; it is necessary in addition to determine the velocities of absorption of both hairbearing and adjacent hairless epidermal cells. In the onion root (**12**) the comparatively larger share of water intake by relatively older root regions expresses itself as a larger capacity for absorption. Relatively high velocities of absorption in the hairless epidermal cells could compensate for lower rates over a larger area of hair surfaces. The new technique combined with that used by the author in onion root studies provides, for the first time, a method for obtaining quantitative data on this question.

Since no measurements of water absorption by the hairless epidermal cells of radish roots are as yet available, it is interesting to compare the velocities of absorption of hairless epidermal cells of the onion root with results obtained on the hair cells in this investigation. Although, in a strict sense, a comparison is not valid since different types of plants and different solutions were used, the comparison indicates that the velocity of absorption by the hair cell falls within the range of velocities exhibited by hairless cells. In the present investigation the maximum velocity observed in a hair cell of the radish root was 31×10^{-4} cu. mm./sq. mm./min. The significance of this value as a maximum remains to be shown as the work on hair absorption has only started. In the onion root the average velocities of absorption from HOAGLAND'S solution by the hairless cells in the vicinity of the regions of elongation and meristem are lower than this value; in regions 45 mm. from the root tip, the velocities of absorption of distilled water may be as high as 84×10^{-4} cu. mm./sq. mm./min. or as low as 4×10^{-4} cu. mm./sq. mm./min. (**13**). Minimum velocities observed in hair cells were 2 to 3×10^{-4} cu. mm./sq. mm./min.

Raphanus sativus is one of the 87 species of plants reported by FARR (**5**) which form hairs in water. Although root hairs develop on radish seedlings in either tap water or moist air, no elongation of an individual hair was observed during the period of immersion in the experiments reported in this paper. Elongation, however, was resumed after the period

of immersion, showing that the inhibition was reversible. This phenomenon is in agreement with observations by other investigators who have noted that reversible inhibition of elongation of root hairs frequently occurs when a seedling is transferred from one medium to another (**6, 17**). The experimental chamber itself served as an excellent culture chamber for the individual seedlings which formed an abundance of parallel straight hairs with uniform diameter at right angles to the root. No abnormalities of growth were observed and rupturing of hair cells rarely occurred except as a result of mechanical injury. There was an absence of visible expansion or contraction of the root hair within the limits of micrometer readings.

The experiments furnish strong evidence for unequal absorption in different areas of the individual cell under the given conditions. This shows that a comparison on the basis of surface is of great importance. It might be that there is also a functional polarity of water absorption along the longitudinal axis of the root hair when totally immersed, with the greatest velocity of absorption at the apical end. Further experimentation is necessary to establish this as a fact. The phenomenon of cellular polarity has a special interest to the general physiologist; no data have been reported heretofore which definitely show that different minute regions of a single cell absorb water at different rates. CHAMBERS and KERR (**2**) found that the wall of the root hair (*Limnobium spongia*) in both living and dead cells was more freely permeable to ammonia at the tip than elsewhere; CHAMBERS and KEMPTON (**3**) have reported a characteristic polarity of the proximal tubules of the chick embryo; the cells absorb phenol red from the external medium on one side and transfer it through the cell and out into the lumen of the tubule on the other side. LUND (**9**) found a distinct polarity in filamentous algal cells with respect to the distribution of bioelectric potentials along the longitudinal axis; the highest E.M.F. appeared at the apical growing point of the apical cell and in the apical regions of adjacent cells.

The data do not show which factors are responsible for the observed differences in velocity exhibited by different regions; on the one hand, they might be due to intracellular differences in metabolic energy or osmotic energy; on the other hand, they might be due to physical characteristics of the limiting membranes. It will be of extreme interest and importance to determine the effect of the presence or absence of oxygen, and the effect of poisoning with narcotics, cyanide, and other respiratory poisons. The technique employed in the present experiments provides for the passage of gases through the sealed chamber and for the absorption of different substances from the micropotometers. It therefore serves as a unique tool to study the factors which regulate the entry of water into the single hair cell.

Of particular interest in connection with determination of the physical or metabolic factors responsible for the differences in velocity of absorption over the surface of an individual root hair observed in the present work is the presence of the nucleus at or near the tip of the radish hair and the

differences in the structure of the cell wall. The structure of the radish root hair has received some attention from CORMACK (**4**) who worked chiefly with varieties of Chinese cabbage but used other plants to test the general applicability of his conclusions. He reports that the wall of a normal hair has a cellulose and a pectic layer continuous with corresponding layers in the epidermal cell; that while the root hair is growing the cellulose layer is soft and easily hydrolyzed and gives every indication of being uniform over the whole surface; that the pectic layer on the sides of the hair is of firm calcium pectate; and the same is true of the parent cell walls. On the dome-shaped tip the layer is composed either of pectic acid or of a softer calcium pectate. In the mature hair the cellulose wall is less easily hydrolyzed and the surface is covered by a layer of calcium pectate of the firmer type. A comparison of the intake of water by young root hairs with that of old root hairs may throw light on numerous problems and since the root hairs of certain water plants (**2**) have nuclei at the base of the hair, they can serve as material for comparative studies on the relation of the nucleus to polarity.

Of further interest is the elucidation of the rôle played by cortical cells adjacent to the hairbearing epidermal cell as well as cells beyond the cortex. In order that continuous water entry might take place in a cell which does not increase in volume there must be a continuous transfer of water to adjacent cells. The speed of absorption may be determined by resistance to passage of water offered by such adjacent cells. The author has developed a method to study this problem.

The technique has a peculiarly significant relation to problems of soil science. Individual micropotometers simulate capillary passages in the soil and this method provides an approach to the problem of determining why different plants do not avail themselves equally of soil moisture.

Summary

1. A microtechnique which may be applied to a quantitative study of the absorption of water and solutes by an individual root hair cell is described.

2. The results furnish for the first time quantitative data in support of the many investigators who have maintained, without direct experimental evidence, that root hairs function in the process of water absorption by higher plants.

3. Measurements were made on the absorption of tap water by individual root hairs of young radish (*Raphanus sativus*) seedlings. The maximum average velocity of absorption observed under the particular conditions described was 31×10^{-4} cu. mm./sq. mm./min.; the minimum was 2×10^{-4} cu. mm./sq. mm./min.

4. The data furnish strong evidence for unequal absorption in different areas of a single hairbearing epidermal cell under the particular conditions described.

5. Attention is called to how the microtechnique described furnishes a unique tool to study factors which regulate the entry of water and solutes into a single cell and in higher plants.

Grateful acknowledgement is made to my technical assistant A. A. HORAK for his aid in this investigation and to PROFESSOR E. P. SCHOCH, of the Bureau of Industrial Chemistry, for tap water analysis.

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EFFECTS OF IRON ON THE GROWTH AND ASH CONSTITUENTS OF *ANANAS COMOSUS* (L.) MERR.[1]

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(WITH ELEVEN FIGURES)

Introduction

Studies on the iron requirements of *Ananas comosus* (L.) Merr. grown under Hawaiian conditions have been made at different times by various investigators (**13, 14**). The consensus of most investigators has been that manganese (**13, 14**) and calcium (**6, 7**) either through the precipitation of water-soluble iron in the soil or in the tissues of the plant were able to cause chlorosis in *A. comosus*. Other conditions, such as different degrees of acidity known either to favor or inhibit the solubility of iron, received at times only little or no attention, probably because methods for measuring H-ion concentrations either electrometrically or colorimetrically had not gained widespread recognition during the first two decades of this century and their application to agricultural problems was at that time in its initial stages.

This paper presents data and a discussion on the effects of "plus-" and "minus-iron" cultures on plant growth and other physiological conditions affecting the values of dry matter, water, ash, electrical resistance, potassium, calcium, magnesium, phosphorus, iron, and manganese in the tissues of *A. comosus* grown in solution cultures supplied either with nitrate or ammonium salts as sources for nitrogen.

The object of growing plants in solution cultures supplied with nitrate or ammonium salts was to study the effects of changes in opposite directions in the H-ion concentration of these solution cultures on the availability of iron to plants. It was observed in former studies that in the course of 2-week intervals H-ion concentrations decreased gradually in nitrate-nitrogen cultures from pH 4.4 to 6.8, and they increased in ammonium-nitrogen cultures from pH 6.6 to 4.2.

Methods

Cultural procedures

Slips (shoots at the apex of vestigial fruits produced laterally on the peduncle) from plants of a single clone of uniform quality and weight were grown for one month in tap water for the formation of roots of approximately uniform length and of equal numbers. They were then transferred to nutrient solution cultures supplied either with nitrate or ammonium salts and other nutrient elements, and either with or without iron, as reported in table I. Each treatment included 16 plants. The various C.P. chemicals employed for the preparation of the nutrient solutions had not been treated

[1] Published with the approval of the Acting Director as Technical Paper no. 146 of the Pineapple Research Institute, University of Hawaii.

in any way to remove the slight contaminations of iron they contained, and our definition of "minus-iron" cultures is subject to the iron content limitations of C.P. chemicals.

The volume of the containers was approximately 18 liters and the solutions were changed at 2-week intervals. The cultures were constantly aerated and the plants were allowed to grow for about one year; that is, as near as possible to the flowering period. Eight plants from each treatment were then harvested, weighed, and sectioned, following the technique reported in a former publication (**23**), and analyzed according to methods reported below.

TABLE I

COMPOSITION OF NUTRIENT SOLUTIONS

Salt	Molar conc.	Elements	Nitrate series		Ammonia series	
			− Fe	+ Fe	− Fe	+ Fe
			mg./l.	*mg./l.*	*mg./l.*	*mg./l.*
$Ca(NO_3)_2$	0.001	N	28.0	28.0	.....	.....
$(NH_4)_2SO_4$	0.001	N	.....	.....	28.0	28.0
K_2SO_4	0.0005	K	58.5	58.5	58.5	58.5
KH_2PO_4	0.0005	P	15.5	15.5	15.5	15.5
$Ca(NO_3)_2$	0.001	Ca	40.0	40.0	.....	.....
$CaCl_2$	0.001	Ca	.....	.....	40.0	40.0
$MgSO_4$	0.001	Mg	24.0	24.0	24.0	24.0
K_2SO_4, $MgSO_4$	0.0015	S	48.0	51.0	.....	.....
K_2SO_4, $MgSO_4$, $(NH_4)_2SO_4$	0.0025	.....	.....	.....	80.0	83.0
$CaCl_2$	0.001	Cl	0.0	0.0	72.0	72.0
$FeSO_4$	0.0001	Fe	.....	5.0	.....	5.0
Mn, B	.....	Micro.	0.5	0.5	0.5	0.5
Zn, Cu, I	.....	Micro.	0.1	0.1	0.1	0.1

CHEMICAL PROCEDURES

For an analysis of the ash constituents, 2 grams of oven-dried plant tissues from a composite sample of 8 plants were ashed in a platinum dish. The ash was dissolved in 100 ml. of an aqueous solution of 1.85 per cent. hydrochloric acid. The mixture was then filtered to remove suspended particles of silica and different aliquots of the filtrate were taken for the determination of the various elements mentioned below.

Potassium was determined by the Nitroso-R-salt colorimetric method (**18**) in a 5-ml. aliquot of the above solution. A 50-ml. aliquot of the solution was used for determining calcium. This was neutralized with 5 N sodium hydroxide using phenolphthalein as indicator, then acidified with 1 ml. of a 50 per cent. solution of acetic acid and subsequently treated with 2 ml. of a 4 per cent. solution of ammonium oxalate. The mixture was allowed to stand overnight, then filtered through asbestos pulp and washed 2 times successively with 20 ml. of water. The precipitate was titrated with 0.05 N potassium permanganate according to the usual procedure and the filtrate was employed for the determination of calcium. An aliquot of the

filtrate from the analysis of calcium, representing 10 ml. of the original volume of the solution, was taken for the determination of magnesium. The 8-hydroxyquinoline colorimetric method (**20**) was employed for the determination of magnesium. Phosphorus was determined as phosphate-phosphorus in a 2-ml. aliquot of the original solution using the method of BERENBLUM and CHAIN (**2**) as modified by the senior author (**22**). The determination of iron was conducted in a 10-ml. aliquot employing the a-a-dipyridyl method of HILL (**11**) and the orthophenanthroline of SAYWELL and CUNNINGHAM (**17**). Only the results obtained by the a-a-dipyridyl method are reported here. The determination of iron with a-a-dipyridyl, as conducted by the authors, deviated in certain respects from the original method. The 10-ml. aliquot was neutralized with NaOH using phenolphthalein as indicator and then treated immediately with 1 ml. of a 20 per cent. solution of glacial acetic acid. The ferric iron was reduced with 2 ml. of a 5 per cent. solution of sodium metabisulphite or pyrosulphite ($Na_2S_2O_5$). It was found that most samples of $Na_2S_2O_5$ gave a very slight positive test for Fe^{++} and it was necessary to run a blank at the same time for correction. Manganese was determined in a 10-ml. aliquot using the improved formaldoxime colorimetric method of the senior author (**21**).

STATISTICAL PROCEDURES

The significance of the difference between the means of the total plant weights of the "plus-iron" and "minus-iron" cultures of the nitrate- and ammonium-nitrogen series was calculated according to FISHER (**4**, p. 114).

The statistical significance of the differences between the amounts of dry matter, ash, potassium, calcium, magnesium, phosphorus, iron, and manganese in various sections of the plants of the various cultures was calculated from CONRAD's (**3**) modification of BESSEL's formula.

The significance of the Z values from the above calculations was obtained from Student's table, as calculated by LOVE (**15**). A summary of the significance of the Z values of the various items is presented in table VIII.

Results

The data concerning plant growth, dry matter, water content, ash and electrical resistance of different sections of the leaves, stem and roots are presented in tables II to V and in figures 1 to 5. Synoptic expressions have been introduced to replace longer ones for certain often repeated appellations. Thus the series nitrate-nitrogen will be designated by N-n and ammonium-nitrogen by A-n. Also the cultures "plus-iron" will be designated by F and "minus-iron" by O.

PLANT WEIGHTS

The data on plant growth in table II and in figure 1 show that the weights of the plants of the F cultures of the N-n series were 33 per cent. greater than those of the plants of the O cultures. The plants of the F and O cultures of the A-n series presented no significant differences in weights.

TABLE II

MEAN, STANDARD DEVIATION, COEFFICIENT OF VARIABILITY, AND PROBABLE ERROR OF THE MEAN OF PLANT AND ROOT WEIGHTS AND OF LENGTHS AND WIDTHS OF THE MATURE (C) AND ACTIVE (D) GROUPS OF LEAVES OF *A. comosus* GROWN IN PLUS- OR MINUS-IRON SOLUTION CULTURES AND SUPPLIED WITH NITRATE SALTS AS SOURCES FOR NITROGEN

	Minus iron				Plus iron			
			Leaf groups				Leaf groups	
	Plant	Roots	Mature (C) L.* W.*	Active (D) L. W.	Plant	Roots	Mature (C) L. W.	Active (D) L. W.
	gm.	*gm.*	*cm.*	*cm.*	*gm.*	*gm.*	*cm.*	*cm.*
M	2510	382	64.0 5.8	80.0 6.4	3315	406	77.0 6.2	94.0 6.8
σ	369	61	8.0 0.4	5.0 0.6	455	83	5.0 0.3	10.0 0.4
CV	15	16	12.0 7.0	5.0 9.0	14	20	6.0 5.0	11.0 6.0
PE	78	13	1.7 0.09	1.1 0.13	93	17	1.0 0.06	2.0 0.08
No. of plants	10	10	10	10	11	11	11	11

* L. = length; W. = width.

This suggests that contaminations of iron in the C.P. nutrient salts were sufficient for growth of the plants under the gradually increasing H-ion concentration of the nutrient solutions of the A-n series, which made all traces of iron available to plants. Calculation of the significance of the difference of the means of the total plant weights, according to the method of FISHER as mentioned above, shows that in the N-n series total weights were very significantly greater (100:1) in the F cultures.

The weights of roots were not as much affected by either the source of nitrogen or amounts of iron as those of the stem and leaves according to the data in tables II and III and in figure 1, and observed differences lacked significance.

TABLE III

MEAN, STANDARD DEVIATION, COEFFICIENT OF VARIABILITY, AND PROBABLE ERROR OF THE MEAN OF PLANT AND ROOT WEIGHTS AND OF LENGTHS AND WIDTHS OF THE MATURE (C) AND ACTIVE (D) GROUPS OF LEAVES OF *A. comosus* GROWN IN PLUS- OR MINUS-IRON SOLUTION CULTURES AND SUPPLIED WITH AMMONIUM SALTS AS SOURCES FOR NITROGEN

	Minus iron				Plus iron			
			Leaf groups				Leaf groups	
	Plant	Roots	Mature (C) L.* W.*	Active (D) L. W.	Plant	Roots	Mature (C) L. W.	Active (D) L. W.
	gm.	*gm.*	*cm.*	*cm.*	*gm.*	*gm.*	*cm.*	*cm.*
M	3176	368	71.0 6.2	86.0 6.7	3151	299	75.0 6.4	83.0 6.6
σ	475	50	6.0 0.6	5.0 0.3	533	64	6.0 0.6	6.0 0.6
CV	15	14	8.0 10.0	6.0 4.0	17	21	8.0 9.0	7.0 9.0
PE	97	10	1.2 0.12	1.0 0.06	128	15	1.4 0.14	1.4 0.14
No. of plants	11	11	11	11	8	8	8	8

* L. = length; W. = width.

Dry matter

The weights of dry matter, reported in table IV and in figure 2, differed for comparable sections of the leaves and stem of the plants of the different treatments. The significance of such differences is discussed in greater detail below. The values of dry matter, depicted in figure 2, show that they

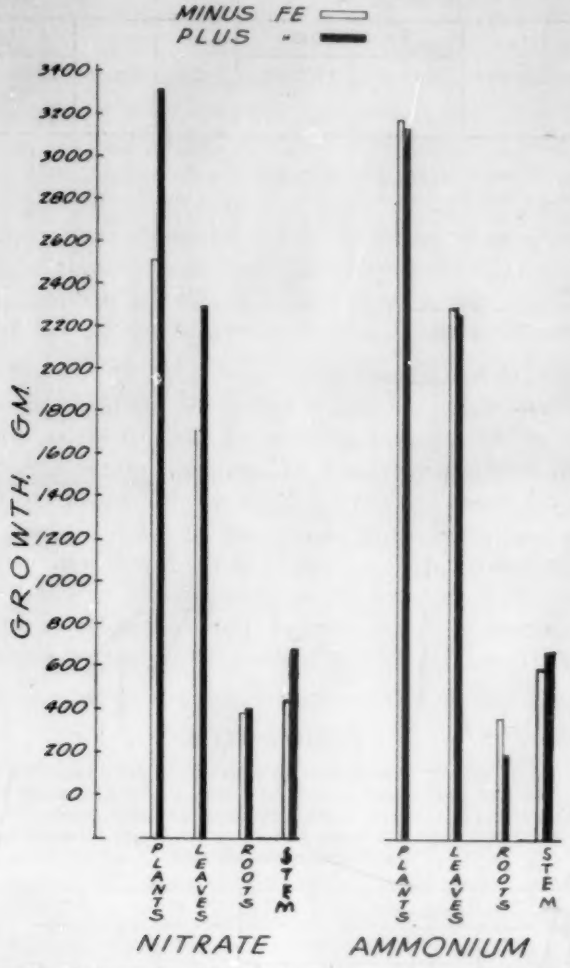

Fig. 1. Growth of pineapple with nitrate and ammonium, with and without iron.

were greater for the F than for the O cultures. Exceptions were the basal (no. 1) and transitional (no. 2) sections of the leaves of the O cultures of the N-n series where the high values of dry matter resulted from decreased tissue succulence through a diminished rate of plant growth. The substances which contributed greatly to the high values of dry matter in plants of the F cultures of both series were sugars, starches, etc., as will be shown in a subsequent publication.

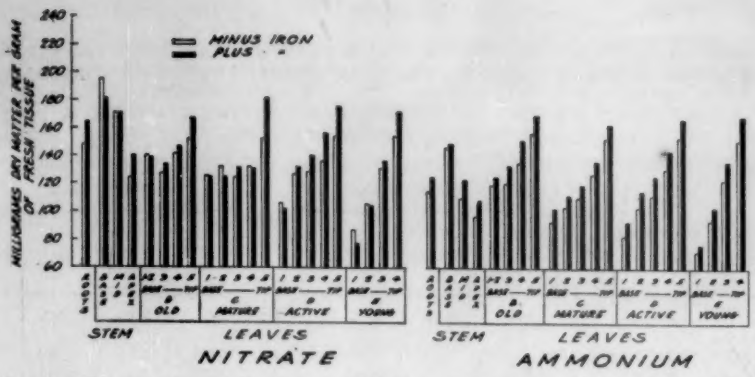

FIG. 2. Dry matter production on nitrate or ammonium, with or without iron.

WATER

The moisture content values of the different sections of the leaves, stem, and roots were inversely proportional to those of dry matter, as shown in table IV and in figure 3. The moisture content was generally higher in

TABLE IV

MILLIGRAMS OF WATER, DRY MATTER, AND ASH PER GRAM OF FRESH TISSUE AND ELECTRICAL RESISTANCE (OHMS) OF THE EXTRACTED SAP IN DIFFERENT SECTIONS OF THE LEAVES, STEM AND ROOTS OF ONE-YEAR-OLD PLANTS OF *A. comosus* GROWN IN PLUS- OR MINUS-IRON SOLUTION CULTURES AND SUPPLIED WITH NITRATE SALTS AS SOURCES FOR NITROGEN

PLANT SECTIONS		MINUS IRON				PLUS IRON			
		WATER	DRY MATTER	ASH	OHMS	WATER	DRY MATTER	ASH	OHMS
Leaves:		*mg.*	*mg.*	*mg.*		*mg.*	*mg.*	*mg.*	
Old (B)	1+2 (base)	860	140	7.70	79.8	861	139	9.32	78.2
	3	873	127	12.57	54.0	866	134	14.60	57.0
	4	859	141	16.90	48.6	853	147	17.05	50.5
	5 (tip)	848	152	18.57	45.6	833	167	17.20	49.8
Mature (C)	1 (base)	875	125	7.12	92.5	878	122	6.10	100.0
	2	868	132	10.18	66.0	877	123	8.86	67.2
	3	876	124	14.90	52.8	869	131	13.36	57.0
	4	868	132	17.95	48.0	869	131	14.92	50.4
	5 (tip)	848	152	20.50	46.8	819	181	18.20	45.0
Active (D)	1 (base)	894	106	6.15	82.2	898	102	6.38	84.0
	2	874	126	8.06	70.8	871	129	8.12	71.4
	3	872	128	11.34	56.4	869	140	10.93	57.0
	4	864	136	15.76	49.2	844	156	13.90	49.8
	5 (tip)	846	154	19.10	43.2	825	175	14.00	44.4
Young (E)	1 (base)	913	87	7.84	72.6	922	78	7.25	66.0
	2	895	105	7.98	78.0	896	104	7.70	77.4
	3	870	130	10.67	60.0	865	135	9.80	63.0
	4+5 (tip)	846	154	14.50	51.0	829	171	11.28	48.6
Stem:									
Base		805	195	15.60	58.2	819	181	14.10	61.8
Middle		829	171	21.90	48.2	829	171	18.95	48.0
Apex		876	124	16.00	52.2	860	140	15.50	52.2
Roots		852	148	4.73	110.0	836	164	7.38	114.0

TABLE V

MILLIGRAMS OF WATER, DRY MATTER, AND ASH PER GRAM OF FRESH TISSUE AND ELECTRICAL RESISTANCE (OHMS) OF THE EXTRACTED SAP IN DIFFERENT SECTIONS OF THE LEAVES, STEM AND ROOTS OF ONE-YEAR-OLD PLANTS OF *A. comosus* GROWN IN PLUS- OR MINUS-IRON SOLUTION CULTURES AND SUPPLIED WITH AMMONIUM SALTS AS SOURCES FOR NITROGEN

PLANT SECTIONS			MINUS IRON				PLUS IRON			
			WATER	DRY MATTER	ASH	OHMS	WATER	DRY MATTER	ASH	OHMS
			mg.	*mg.*	*mg.*		*mg.*	*mg.*	*mg.*	
Leaves:										
Old (B)	1+2	(base)	881	119	7.55	93.0	876	124	7.44	82.2
	3		880	120	11.66	61.8	868	132	12.94	57.6
	4		865	135	11.34	57.6	849	151	12.55	54.6
	5	(tip)	844	156	10.92	55.2	831	168	12.00	51.6
Mature (C)	1	(base)	907	93	5.93	97.8	898	102	6.23	87.6
	2		897	103	9.58	68.4	889	111	9.10	62.4
	3		890	110	11.00	61.2	881	119	11.11	55.2
	4		874	126	12.36	55.8	864	136	13.06	51.6
	5	(tip)	848	152	11.10	54.6	838	162	14.30	52.2
Active (D)	1	(base)	917	83	6.16	93.0	907	93	6.13	84.0
	2		897	103	7.00	73.8	886	114	7.18	69.6
	3		889	111	10.54	56.4	875	125	11.76	52.2
	4		870	130	12.50	51.6	856	144	13.52	49.8
	5	(tip)	847	153	10.25	51.0	834	166	15.93	48.6
Young (E)	1	(base)	928	72	6.40	82.2	923	77	6.78	73.8
	2		906	94	7.23	78.0	897	103	5.77	74.4
	3		878	122	9.27	60.0	865	135	9.73	62.4
	4+5	(tip)	849	151	10.12	51.6	832	168	8.56	52.2
Stem:										
Base			854	146	11.23	72.6	850	150	9.95	63.0
Middle			891	109	16.10	52.2	878	122	13.90	51.0
Apex			903	97	12.12	56.4	892	108	11.88	55.2
Roots			886	114	2.39	115.0	876	124	5.70	85.8

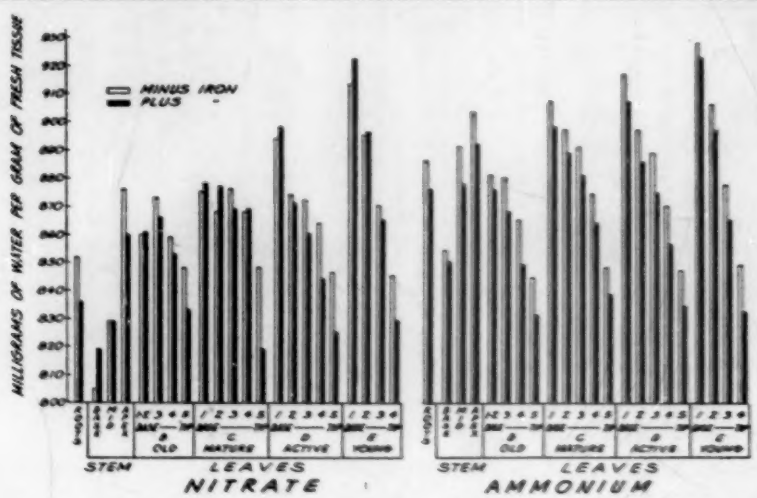

FIG. 3. Water content of tissues grown with nitrate or ammonium, and with or without iron.

sections containing meristematic tissues than in tissues of advanced chronological and physiological age. Moisture content values were greater in most plant sections from the O than F cultures of both series. Exceptions were the basal sections of leaves and apical ones of the stem of the N-n series.

The moisture content was generally higher for the plants of the A-n than of the N-n series, a phenomenon also observed in former studies (**23**).

Ash

The ash values, reported in table V and in figure 4, were generally higher for the plants of the N-n than of the A-n series. Differences between the ash content of the F and O cultures were not generally consistent. Many of the leaf and stem sections from the O cultures of the N-n series contained

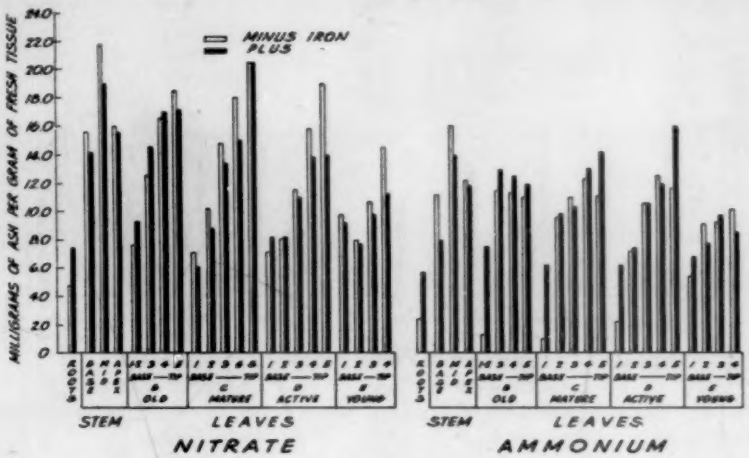

FIG. 4. Ash content of tissues from plants grown on nitrate or ammonium, with or without iron.

more ash than comparable sections from the F cultures of the same series. This might have resulted from so-called concentration effects, that is, from salt accumulations caused by differences in the weights of the plants of the different treatments rather than from differences in the rates of ash constituents entering their tissues. The results obtained from the A-n series indicate that slightly greater concentrations of ash-forming elements had accumulated in the tissues of most sections of the plants of the F than of the O cultures. Evidence (below) suggests that ammonium ions (NH_4^+) decrease or retard the absorption of other cations, especially of calcium, which might account for the generally lower values of ash in the plants of the A-n series.

Electrical resistance

The values of the relative electrical resistance (ohms) of the extracted sap of the various sections, reported in tables IV and V and in figure 5, were higher, with a few exceptions, in the sections of the plants of the F

than O cultures of the N-n series. This relationship was reversed in the A-n series. Electrical resistance values were approximately inversely proportional to ash values for comparable plant sections, as shown in tables IV and V. Owing to the fact that such values may be increased by weak electrolytes such as carboxylic acids or amino acids which occur in various amounts in different plant sections, or decreased by non-electrolytes such as sugars through interference of ionic movements, they are of limited value for predicting total ash values because they represent the sum total of the conductance of all inorganic and organic electrolytes and non-electrolytes.

Potassium

The data reported in tables VI and VII and in figure 6 show that the plants of the O cultures of the N-n series contained, with few minor exceptions, slightly higher amounts of potassium than those of the F cultures of the same series. Moreover, they suggest that the rate of potassium absorption by the plants of the O cultures of the N-n series remained constant, while that of growth was appreciably reduced by the lack of adequate amounts of iron, resulting in a greater concentration of potassium per plant volume. The reverse of this condition occurred, with the exception of a few terminal (no. 5) leaf sections, in the plants of the O cultures of the A-n series which contained slightly smaller amounts of potassium than those of the F cultures. Comparing the amounts of potassium in the plants of the F cultures between the N-n and A-n series, we observe that they were slightly greater in many sections of the plants of the latter than former series. The concentration gradient of potassium between the basal and the terminal sections of the leaves varied from 200 to 400 per cent. in different groups of leaves, whereas in the stem it varied relatively little.

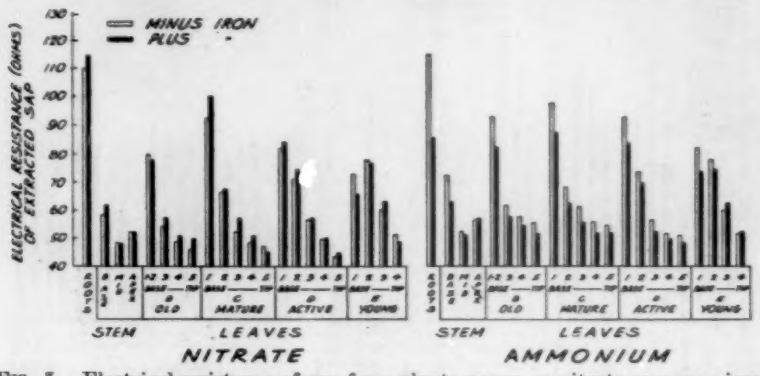

Fig. 5. Electrical resistance of sap from plants grown on nitrate or ammonium, with or without iron.

Calcium

The differences in the amounts of calcium between the plants of the O and F cultures of the same nitrogen series were relatively small as reported

TABLE VI

MILLIGRAMS OF POTASSIUM, CALCIUM, MAGNESIUM, AND PHOSPHORUS, AND MICROGRAMS OF IRON AND MANGANESE PER GRAM OF FRESH TISSUE IN DIFFERENT SECTIONS OF THE LEAVES, STEM AND ROOTS OF ONE-YEAR-OLD PLANTS OF *A. comosus* GROWN IN PLUS- OR MINUS-IRON SOLUTION CULTURES AND SUPPLIED WITH NITRATE SALTS AS SOURCES FOR NITROGEN

Plant sections		Minus iron						Plus iron					
		K	Ca	Mg	P	Fe	Mn	K	Ca	Mg	P	Fe	Mn
Leaves:		*mg.*	*mg.*	*mg.*	*mg.*	γ	γ	*mg.*	*mg.*	*mg.*	*mg.*	γ	γ
Old (B)	1 + 2 (base)	5.84	0.26	0.14	0.20	0.22	0.45	6.43	0.30	0.10	0.09	0.43	0.45
	3	8.44	0.51	0.38	0.26	0.18	0.37	8.75	0.64	0.46	0.13	0.50	0.35
	4	9.93	0.89	0.62	0.28	0.20	0.79	9.18	0.93	0.66	0.17	0.61	0.80
	5 (tip)	11.85	0.90	1.12	0.29	0.35	1.41	8.82	1.31	0.97	0.22	0.77	0.95
Mature (C)	1 (base)	4.47	0.33	0.17	0.06	0.18	0.18	3.82	0.46	0.11	0.08	0.44	0.37
	2	8.25	0.27	0.11	0.13	0.15	0.58	8.06	0.31	0.06	0.13	0.33	0.30
	3	10.37	0.32	0.31	0.17	0.16	0.87	8.95	0.50	0.23	0.14	0.38	0.29
	4	12.00	0.78	0.62	0.21	0.22	1.18	8.95	0.63	0.39	0.14	0.59	0.43
	5 (tip)	14.60	0.78	0.76	0.25	0.20	2.43	12.12	1.09	0.65	0.22	0.86	0.94
Active (D)	1 (base)	3.49	0.26	0.16	0.05	0.12	0.17	3.82	0.43	0.20	0.07	0.34	0.16
	2	5.24	0.21	0.12	0.08	0.14	0.25	5.97	0.36	0.11	0.11	0.41	0.21
	3	8.00	0.31	0.25	0.11	0.14	0.36	7.62	0.45	0.19	0.11	0.44	0.22
	4	11.34	0.41	0.39	0.14	0.15	0.82	8.62	0.54	0.29	0.11	0.49	0.41
	5 (tip)	13.32	0.67	0.54	0.21	0.20	1.66	9.67	0.67	0.44	0.13	0.50	0.69
Young (E)	1 (base)	4.57	0.20	0.33	0.05	0.17	0.16	4.88	0.26	0.27	0.07	0.28	0.27
	2	5.14	0.18	0.21	0.06	0.16	0.27	4.88	0.29	0.21	0.08	0.31	0.25
	3	6.92	0.23	0.25	0.10	0.22	0.31	6.40	0.36	0.22	0.09	0.35	0.38
	4 + 5 (tip)	9.62	0.42	0.45	0.14	0.32	0.99	8.10	0.59	0.50	0.10	0.48	0.64
Stem:													
Base		4.76	2.65	0.67	0.07	0.35	0.43	5.55	2.46	0.60	0.09	0.52	0.47
Middle		7.12	3.42	0.75	0.20	0.22	0.44	6.41	3.42	0.43	0.12	0.46	0.41
Apex		7.32	1.71	0.67	0.18	0.20	0.32	7.00	2.24	0.44	0.14	0.40	0.31
Roots		2.37	0.24	0.22	0.05	1.17	0.38	1.64	0.45	0.32	0.08	21.90	0.61

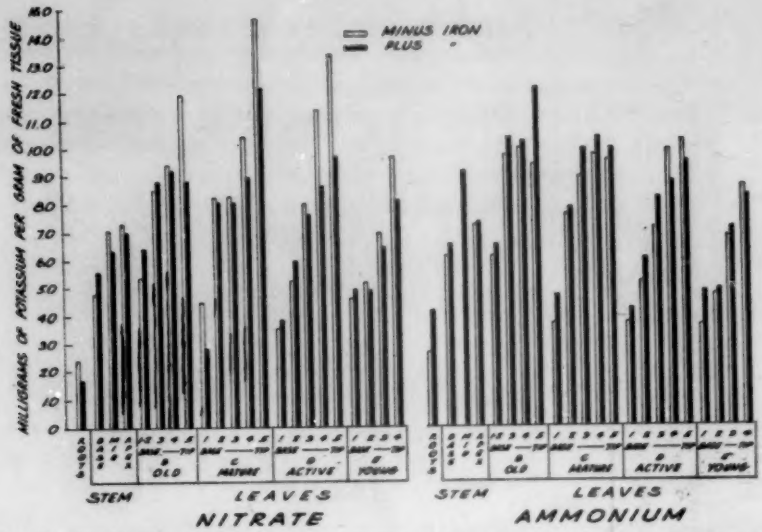

FIG. 6. Potassium content of tissues from plants grown with nitrate or ammonium, with or without iron.

in tables VI and VII and in figure 7. However, those between different nitrogen series, namely between the A-n and N-n series but of comparable iron cultures, were relatively great. The calcium content of the stem in the

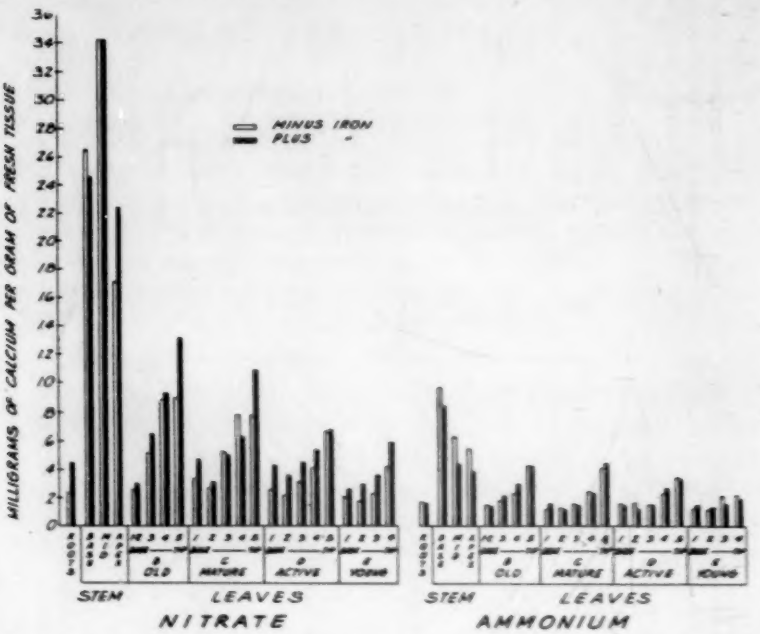

FIG. 7. Calcium content of tissues from plants on nitrate or ammonium, with or without iron.

TABLE VII

MILLIGRAMS OF POTASSIUM, CALCIUM, MAGNESIUM, AND PHOSPHORUS AND MICROGRAMS OF IRON AND MANGANESE PER GRAM OF FRESH TISSUE IN DIFFERENT SECTIONS OF THE LEAVES, STEM, AND ROOTS OF ONE-YEAR-OLD PLANTS OF *A. comosus* GROWN IN PLUS- OR MINUS-IRON SOLUTION CULTURES AND SUPPLIED WITH AMMONIUM SALTS AS SOURCES FOR NITROGEN

PLANT SECTIONS		MINUS IRON						PLUS IRON					
		K	Ca	Mg	P	Fe	Mn	K	Ca	Mg	P	Fe	Mn
Leaves:		*mg.*	*mg.*	*mg.*	*mg.*	γ	γ	*mg.*	*mg.*	*mg.*	*mg.*	γ	γ
Old (B)	1+2 (base)	6.07	0.15	0.31	0.15	0.24	0.30	6.52	0.15	.....	0.14	0.37	0.12
	3	9.67	0.18	0.36	0.19	0.27	0.63	10.30	0.21	0.22	0.37	1.20	0.33
	4	9.94	0.23	0.81	0.30	0.38	0.64	10.20	0.29	.....	0.35	1.60	0.66
	5 (tip)	9.33	0.42	0.90	0.37	0.52	2.16	12.08	0.42	0.44	0.42	1.49	1.08
Mature (C)	1 (base)	3.72	0.12	0.67	0.08	0.22	0.16	4.64	0.16	0.17	0.10	0.42	0.10
	2	7.58	0.13	0.32	0.19	0.23	0.16	7.83	0.12	.....	0.09	0.47	0.17
	3	8.87	0.16	0.41	0.23	0.29	0.30	9.93	0.15	0.14	0.19	0.62	0.24
	4	9.68	0.24	1.18	0.26	0.32	0.41	10.30	0.22	0.17	0.24	1.02	0.51
	5 (tip)	9.50	0.41	1.08	0.36	0.35	1.41	9.90	0.45	0.59	0.30	1.66	0.93
Active (D)	1 (base)	3.70	0.16	0.40	0.07	0.21	0.10	4.23	0.15	0.15	0.07	0.34	0.09
	2	5.15	0.17	0.29	0.10	0.23	0.13	6.00	0.12	0.08	0.08	0.46	0.12
	3	7.12	0.15	0.38	0.15	0.25	0.11	8.18	0.15	0.16	0.08	0.50	0.16
	4	9.86	0.23	0.54	0.22	0.24	0.20	8.68	0.27	0.25	0.16	0.66	0.29
	5 (tip)	10.20	0.34	0.77	0.23	0.38	0.50	9.43	0.34	0.25	0.17	1.08	0.63
Young (E)	1 (base)	3.60	0.13	0.40	0.06	0.18	0.13	4.81	0.15	0.29	0.05	0.33	0.08
	2	4.70	0.12	0.28	0.07	0.22	0.16	4.90	0.13	0.19	0.06	0.40	0.13
	3	6.78	0.21	0.61	0.11	0.26	0.24	7.10	0.16	0.25	0.08	0.57	0.20
	4+5 (tip)	8.57	0.22	0.61	0.11	0.35	0.32	8.25	0.19	0.33	0.09	0.67	0.42
Stem:													
Base		6.08	0.97	1.08	0.13	0.31	0.15	6.52	0.85	0.31	0.14	0.35	0.19
Middle		9.00	0.64	1.82	0.20	0.23	0.16	9.10	0.44	0.94	0.14	0.43	0.12
Apex		7.22	0.54	2.02	0.14	0.24	0.13	7.30	0.39	0.98	0.12	0.35	0.11
Roots		2.66	0.16	0.45	0.04	0.33	0.29	4.13	0.16	0.32	0.07	28.20	0.50

N-n series was from 2.5 to 5.5 times greater than that in the A-n series. The leaves of the F cultures contained more calcium than those of the O cultures in the N-n series. The results generally emphasize the fact that the absorption of calcium by *A. comosus* was greatly accelerated by nitrate ions and retarded by ammonium ions, while the extra amounts of iron in the F cultures increased calcium absorption only slightly over those of the O cultures. The data suggest that the acceleration of calcium absorption by nitrate ions and its retardation by ammonium ions could have been caused by electrostatic forces as postulated in the theory of GIRARD (**7**) and HAYNES (**10**). In conformity, then, with the above theory of absorption the NO_3^-

TABLE VIII

STATISTICAL SIGNIFICANCE OF THE DIFFERENCE* BETWEEN CONSTITUENTS OF COMPARABLE SECTIONS OF PLANTS GROWN IN PLUS- AND MINUS-IRON CULTURES SUPPLIED EITHER WITH NITRATE OR AMMONIUM SALTS AS SOURCES FOR NITROGEN†

Constituents	Nitrate-N				Ammonium-N			
	Leaves		Stem		Leaves		Stem	
	Approximate significance	In favor of culture	Significance	In favor of culture	Significance	In favor of culture	Significance	In favor of culture
Dry matter	300 : 1	+ Fe	None	.....	9999 : 1	+ Fe	25 : 1	+ Fe
Ash	500 : 1	− Fe	None	.....	40 : 1	+ Fe	None	.....
Potassium	600 : 1	− Fe	None	.....	180 : 1	+ Fe	40 : 1	− Fe
Calcium	9999 : 1	+ Fe	None	.....	None	.....	85 : 1	− Fe
Magnesium	2000 : 1	− Fe	19 : 1	− Fe	9999 : 1	− Fe	260 : 1	− Fe
Phosphorus	250 : 1	− Fe	None	.....	None	.....	None	.....
Iron	9999 : 1	+ Fe	25 : 1	+ Fe	9999 : 1	+ Fe	None	.....
Manganese	250 : 1	− Fe	None	.....	None	.....	None	.....

* Calculated by CONRAD'S formula and from LOVE'S table.

† The significance of the difference (represented by the algebraic sum of the differences of items between comparable plant sections from different treatments) is not used in the strict sense of statistics but as a synoptic index of all such differences.

in the N-n series was electrostatically attracted by Ca^{++} and possibly by other cations, while that of Ca^{++} in the A-n series was electrostatically repelled by NH_4^+ and possibly other cations. It is difficult, however, to explain on the basis of the effects of NO_3^- and NH_4^+ on calcium absorption, why the absorption of potassium and, to a certain degree, of magnesium by roots was not affected in a similar manner in conformity with the above mentioned electrostatic theory. Calcium accumulated in the stem in very great proportions with most of it located in the tissues of the stele (**24**). This element did not move as freely as potassium from the tissues of the stem to the leaves, as indicated by data showing that the stem contained from 2 to 5 times more calcium than the leaves, while potassium accumulated mostly in the terminal sections of the latter organs. The causes for the low rates of calcium translocation from the stem to the leaves are possibly

anatomical and compare well with rates usually attributed to diffusion. Moreover, the accumulation of calcium in the apical tissues of the stem before the flowering period and its depletion thereafter indicate an exceedingly slow movement of this element through the meristematic tissues of the stem apex during the above period which, however, changes very rapidly as soon as the meristematic tissues of the apical region of the stem undergo differentiation during the period of fructification. This postulation has been supported by some anatomical evidence which shows that the apical tissues of the stem and those of the leaf bases lack, during their meristematic stages, well defined and adequately functioning tracheids. In regions of the stem which have advanced in development beyond the meristematic stage, as in the medial region, the conduction of substances from

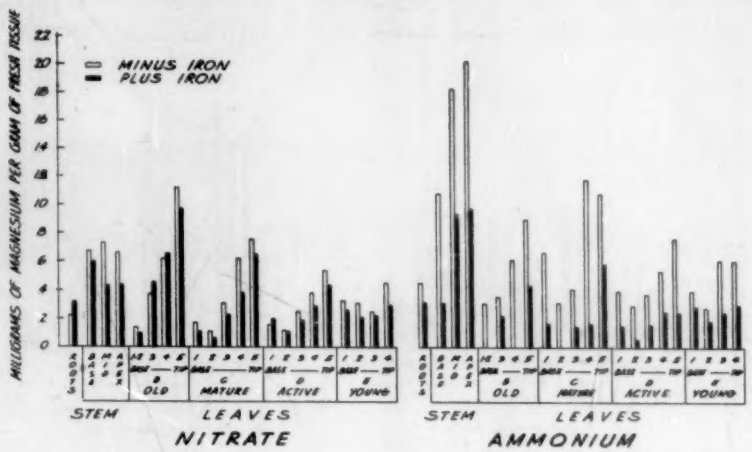

FIG. 8. Magnesium content of tissues from plants grown with nitrate or ammonium, with or without iron.

the tissues of the stem to those of the leaves is more rapid than in the apical region before the flowering period, suggesting that the movement of substances is not dependent on diffusion forces but that it is carried on through well-formed tracheids. The amount of anastomosis between the tracheids of the stem and those of the basal sections of the leaves has not been definitely ascertained.

MAGNESIUM

The distribution of magnesium in different sections of the plants of the various cultures, reported in tables VI and VII and in figure 8, shows that the plants of the O cultures of both nitrogen series contained, with few exceptions, greater quantities of this element than the plants of the F cultures. The plants of the A-n series of the O cultures contained greater amounts of magnesium than those of the corresponding cultures of the N-n series. In the F cultures the average values of magnesium were greater in the leaves of the plants of the N-n than of the A-n series, but in the stem

they were reversed. Magnesium accumulated, although not to the same extent, in the stem of the plants of all treatments. The much greater accumulations of magnesium in the stem of the plants of the O cultures of the A-n series than in any of the other cultures cannot be attributed to concentration effects resulting from differences in plant weights because the plants of the A-n series weighed approximately the same or more than those of the N-n series of comparable iron treatments.

Phosphorus

The distribution of phosphorus in the plants of the various cultures, reported in tables VI and VII and in figure 9, shows that it accumulated in greater amounts in the leaves of the old (B) and mature (C) groups of the plants of the A-n than of the N-n series. The differences between the amounts of phosphorus in the leaves of the active (D) and young (E)

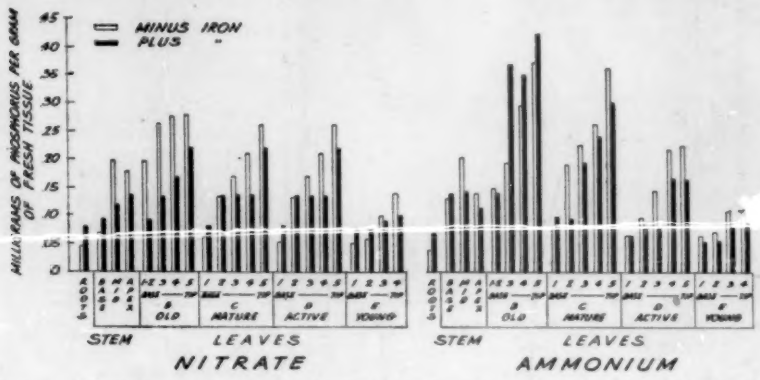

Fig. 9. Phosphorus content of tissues from plants grown on nitrate or ammonium, with or without iron.

groups of the plants of the two nitrogen series were small and irregularly distributed. Many of the sections of the plants of the O cultures of both nitrogen series contained appreciably greater amounts of phosphorus than comparable sections of the plants of the F cultures.

The amounts of phosphorus in roots, although generally very small, were higher for those in the F than in the O cultures of both nitrogen series. It is possible that the slightly greater amounts of phosphorus in the roots of the F than O cultures resulted from the precipitation of some phosphate-phosphorus by iron on the surface. A similar possibility is also suggested by the analytical data on iron.

Iron

The distribution of total iron in the plants of the different treatments and especially in those of the O cultures is very interesting. It was mentioned earlier that iron, in concentrations of 5 mg. per liter of nutrient solution, was added only to the F but not to the O cultures.

The data in tables VI and VII and in figure 10 show that the amounts of iron were generally higher in the plants of the F than of the O cultures. They also show that the plants of the A-n series contained more iron than those of the N-n series. The highest values of iron were found in the roots of the plants of the F cultures of both nitrogen series. The roots of the N-n series of the O cultures contained more iron than those of the corresponding iron treatment of the A-n series. The causes for the accumulation of greater amounts of iron in the stem and leaf tissues of the plants of the A-n than N-n series could be attributed to differences in the availability of iron caused by changes in the H-ion concentration of the solution cultures of the two series. The H-ion concentrations of the cultures of the A-n series (supplied with ammonium sulphate) changed in the course of

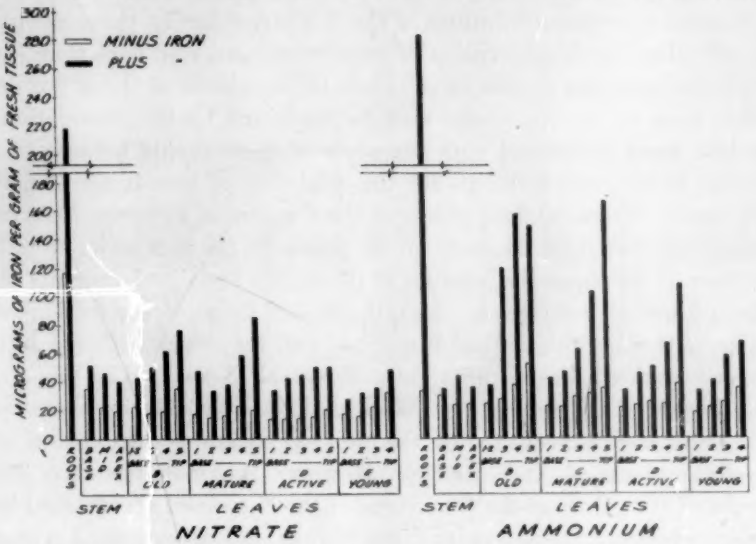

FIG. 10. Iron content of tissues from plants grown on nitrate or ammonium, with or without iron.

two weeks from an initial value of pH 6.6 to a final one of 4.2, and in those of the N-n series (supplied with potassium or calcium nitrate or with both) from pH 4.4 to 6.8. The changes in the initial H-ion concentrations were caused, in the case of the cultures of the A-n series, by a greater rate of absorption of ammonium than of sulphate ions and in that of the N-n series by a greater one of nitrate than of calcium and possibly other cations. The H-ion concentration changes from pH 6.4 to 5.0 in the cultures of the A-n series were relatively rapid, effected mostly in the course of 3 days, and pH values ranging between 4.0 and 5.0 were maintained for the greater portion of the two-week period which favored iron solubility. The results suggest that a great portion of the water-soluble iron of the cultures of the N-n series was possibly precipitated from the solution during the changes in the H-ion concentration from pH 4.4 to 6.4, which condition decreased also the availability of this element to the plants.

The relatively great amounts of iron found in the roots of the plants of the F cultures, as reported in tables VI and VII, resulted, in the opinion of the writers, from iron which was deposited on the surface of the roots during the processes of absorption. Partial confirmation of the precipitation of iron on the superficial tissues of the roots was obtained by suspending well-washed, intact roots in a 2 per cent. solution of sodium hydrosulphite for 4 hours and then analyzing the solution for iron. The availability of iron precipitated on the surface of the roots at favorable H-ion concentrations can be demonstrated if chlorotic plants of *A. comosus* containing such iron on their roots are placed in solution cultures supplied with ammonium sulphate instead of calcium nitrate, or if the pH of the solution is maintained at constant pH values of 4.0 to 5.0. It is also possible that some iron was precipitated in nutrient solutions of the N-n series during the generation of high pH values by the absorption of greater amounts of anions than cations. The greater amounts of iron in the roots of the plants of the F cultures of the A-n than of the N-n series may be explained by the postulation that more iron came in contact with the roots of these plants because of more favorable H-ion concentrations for the solubility of iron in the A-n series. In the case of the roots of the plants of the O cultures, however, the amounts of iron precipitated on the roots of the plants of the A-n series were lower than those of the N-n series because of the highly restricted amounts of iron in the cultures of both series. Also, the higher H-ion concentrations of the cultures of the A-n than of the N-n series, and the scarcity of iron in the O cultures prevented the precipitation and possible deposition of this element on the surface of the roots of the plants of the A-n series, whereas some precipitation of iron was effected by the high pH values of the cultures on the roots of the plants of the N-n series. However, the precipitation of iron on the roots of the plants of the O cultures of the N-n series represented by the differences between the amounts of iron in the O cultures of the A-n and N-n series ($1.17 - 0.33 = 0.84$) demonstrates the efficacy of high pH values on iron precipitation even in solution cultures where the concentrations of this element could not have been any higher than 10^{-8} gm. per liter of solution. Therefore, the absorption of iron by roots may be conditioned in part either by the initial acidity of the nutrient solution or by such subsequent changes in the acidity that develop on the surface of the roots during the absorption of nutrient elements. The precipitation of iron on the surface of the roots of the plants of the O cultures of the N-n series is a paradoxical phenomenon because the quantities of this element, occurring as mere contaminations in the C.P. nutrient salts, were exceedingly small. The precipitation of this element under the conditions of the N-n cultures, however, indicates its great susceptibility to insolubleness and hence its low rate of permeability into the tissues of the roots. The possible precipitation of some iron by phosphorus has been suggested by the results of the chemical analyses.

MANGANESE

The distribution of manganese in the plants of the various treatments is reported in tables VI and VII and in figure 11. The data show that manganese occurred, in the majority of cases, in much greater amounts in the plants of the N-n than of the A-n series. It was generally higher in the stem and leaves but not in the roots of the plants of the O cultures of the N-n series. The smaller amounts of manganese in the leaves and stem of the plants of the F than of the O cultures of the N-n series may be attributed to iron precipitated on the surface of the roots which presumably reduced the rate of manganese permeability into the root tissues. With the amounts of both iron and manganese being greater in the roots of the plants of the

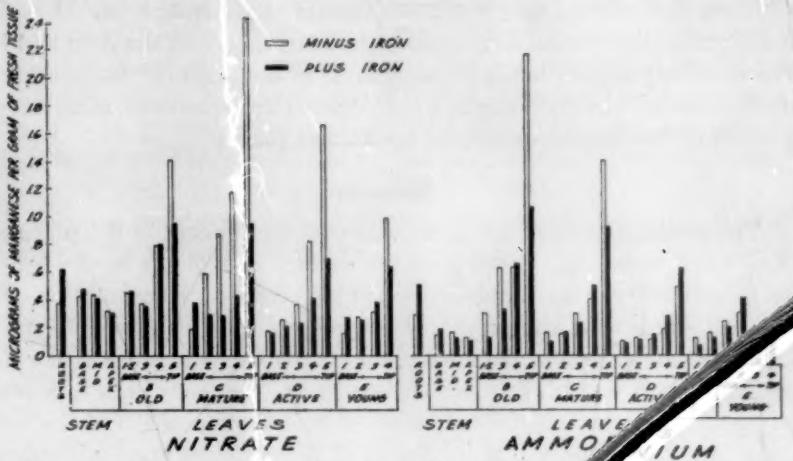

FIG. 11. Manganese content of tissues from plants grown on nitrate or ammonium, with or without iron.

F than of the O cultures, while those of the latter element were reversed in the sections of the leaves and stem, the data suggest that manganese was probably precipitated, like iron, on the surface of the roots.

Analyses of the data in tables VI and VII show that the effects of different pH reactions, as mentioned above for nutrient cultures supplied either with ammonium sulphate or calcium nitrate salts, caused depositions of manganese on the roots of the plants of the O cultures of 0.38 and 0.29 microgram of Mn per gram of fresh tissue for the N-n and A-n series, respectively, equivalent to 23.7 per cent. $\left(\frac{0.38-0.29}{0.38}\times 100\right)$ in favor of the N-n series. The amounts of manganese depositions in the roots of the plants of the F cultures were 0.61 and 0.50 microgram per gram of fresh tissue for for the N-n and A-n series, respectively, equivalent to 18.0 per cent. $\left(\frac{0.61-0.50}{0.61}\times 100\right)$ in favor of the N-n series. The effects of iron on the deposition of Mn in the roots of the plants of the A-n series were 0.50 and

0.29 microgram per gram of fresh tissue for the F [illegible] cultures, respectively, equivalent to 42.0 per cent. $\left(\frac{0.50-0.29}{0.50}\times 100\right)$ in favor of the F cultures. In the plants of the N-n series the Mn depositions in the roots were 0.61 and 0.38 microgram per gram of fresh tissue for the F and O cultures, respectively, equivalent to 46 per cent. $\left(\frac{0.61-0.38}{0.61}\times 100\right)$ in favor of the F cultures. Therefore, the precipitation of Mn caused by the iron content of the F *vs.* the O cultures was greater ($42+46\div 2=44$ per cent.) than that by the different pH values of the cultures of the N-n *vs.* A-n series ($23.7+18.0\div 2=20.8$ per cent.).

The data demonstrate also that the relative (low) acidity of the nutrient solutions had affected only the precipitation of manganese on the roots but it did not interfere with its movement into the tissues of the stem and leaves. The relatively higher values of manganese in the plants of the O cultures of the N-n series, however, might have resulted from concentration effects on account of the smaller weights of the former plants.

Discussion

The results presented above indicate that the changes in H-ion concentrations which constantly develop on the surface of roots or at the regions of the rhizosphere during the absorption of ions from nutrient solution cultures supplied either with nitrate or with ammonium salts as sources for nitrogen may facilitate or inhibit the absorption of iron by roots. The H-ion concentration changes resulting from such conditions may vary both in magnitude and in duration as they depend on the chemical composition of the nutrient solution and on the length of the period of absorption of certain ions by roots. In view of the fact that iron solubility in nutrient solutions and possibly in soils, and its availability to *A. comosus* depends on a favorable range of hydrogen ion concentrations of the nutrient solution and of soils, an evaluation of both pH and water-soluble iron constitutes the only reliable criterion for inorganic iron availability. The generation of acidic and basic reactions at the rhizosphere through the absorption by plant roots of anions and cations at different rates influences the solubility of iron at the point of its entrance into the roots. Any conditions interfering with the solubility of iron in the solution culture or soil render meaningless any comparisons between the initial amounts of iron in the nutrient solution and those absorbed by plants.

The amounts of iron in the basal (1) sections of leaves (the first point of entrance of this element into the leaves from the stem) of the O cultures were slightly smaller in the N-n than A-n series. In spite of the lack of appreciable differences in the concentrations of iron in the basal (1) sections of the young leaves (E) of the O cultures between the N-n and A-n series the difference in chlorophyll between the two series were very great as will be discussed further in a subsequent publication. In the remaining sections

of all leaves of the O and F cultures the amounts of iron were generally higher in the plants of the A-n than N-n series. This indicates that the movement of iron into the leaves continued probably for a longer period and was possibly of a greater intensity in the A-n than N-n series. These findings conform with the postulation advanced above that the quantities of available iron decrease rapidly in solution cultures supplied with nitrate salts (calcium or potassium nitrate) because of the generation of H-ion concentrations (high pH) incompatible with the solubility of iron.

OLSEN (**16**) has suggested the possibility of iron precipitation as iron phosphate within the water-conducting vessels of *Zea mays* L. plants, and WADLEIGH *et al.* (**26**) have extended OLSEN's hypothesis to include the effect of the pH of the sap on iron solubility. The presence of oxidizing agents for the precipitation of iron in plant tissues has also been cited by others (**6**). In order to obtain some idea of the influence of the relative acidity and reducing power of the sap on iron solubility, determinations of both titratable acidity and H-ion concentration and of ascorbic acid were made. These will be reported in a subsequent publication. They suggest, however, that iron in the leaves is susceptible neither to precipitation nor to oxidation from a lack of sufficient acidity or of reducing substances.

The data on the distribution of iron in the sections of the plants of the O cultures of the A-n series, which produced weights almost as great as those of the F cultures, suggest that the absolute iron requirements of *A. comosus* under highly favorable conditions for iron solubility are relatively small (approximately 10^{-7} mg. per liter of solution). They also demonstrate that plant growth, although favored more by the addition of somewhat greater amounts of iron than the mere traces occurring in the O cultures derived from impurities in the C.P. salts, is not affected by excessive amounts, and that such amounts are of no additional value to plants except in nutrient solutions which are subject to pH changes from low to high levels as in those supplied with nitrate salts. Definite limits for iron requirements cannot be set on the basis of plant tissue analyses because the iron content of such tissues is influenced by concentration or dilution effects caused by various complex factors, such as plant growth rate and age, besides the readily available iron content of the substratum.

Our knowledge of the physiological functions of iron in plants is limited except for its catalytic properties in the formation of chlorophyll, or more probably of proto-chlorophyll, and its participation in the composition of catalase and certain cytochromes (**1**). The facts obtained so far suggest that, as long as plants are supplied with iron in concentrations sufficient to catalyze the synthesis of the required amounts of chlorophyll, any greater quantities that may be absorbed by the roots are possibly of no additional physiological value. The fact that greater amounts of iron are often found in the tissues of chlorotic than of green plants constitutes an anomaly and a paradox. It is assumed that such accumulations of iron in the tissues of chlorotic plants result from amounts translocated there after such tissues

ceased to grow and a great deal of their initial metabolic activity and possibly the power of forming more chlorophyll has been appreciably reduced. In all such chlorotic plants the new or meristematic tissues rather than the chronologically and physiologically more advanced tissues of the plants contain less iron than those of non-chlorotic or green plants. Amounts of chlorophyll rather than of iron in plant tissues should be the criteria for evaluating plant response to iron treatments. As the quantities of chlorophyll are also influenced by those of available nitrogen, any deficiencies of the latter are likely to reduce considerably the amounts of chlorophyll in the leaves. Reductions of chlorophyll amounts below certain minimum values directly affect the carbohydrate-synthesizing efficiency of the plant. Thus any deficiencies of iron or nitrogen causing reductions in chlorophyll values influence the rate of plant growth, carbohydrate synthesis, assimilation, nutrient element absorption, and accumulation. It is possible that all such conditions had developed in the plants of the O cultures of the N-n series and caused the production of small plant weights, some accumulations of iron and other elements, and various other physiological disturbances.

The data on the distribution of ash show that the plants of the O cultures of the N-n series contained more of these substances than those of the other cultures because of concentration effects resulting from the smaller weights of the plants of the O cultures. The amounts of ash in the leaves of the F cultures of the A-n series were greater than those of the O cultures, suggesting a greater rate of absorption of ash constituents by the plants of the F than of the O cultures. The amounts of ash in the stem of the O cultures were reversed, suggesting a reduced rate of translocation. In view of the fact that the weights of all the plants of the A-n series were about the same, the greater amounts of ash constituents in the leaves of the F than of the O cultures should be attributed to their greater content of chlorophyll and its effects on the translocation of solutes and normal physiological functioning of the plants.

The distribution of phosphorus shows that slightly greater amounts of this element were deposited in the roots of the plants of the F than of the O cultures of both nitrogen series, suggesting a possible precipitation of both elements as insoluble ferric phosphate.

The formation of other insoluble compounds of iron and phosphorus is suggested by the results of the chemical analyses. The calculations in table IX are an attempt to show certain possible relationships between phosphorus and iron which might have affected their distribution in the roots of the plants of the different cultures.

The calculations in table IX show that the ratio of iron to phosphorus in the F cultures was 0.278 and 0.415 in the N-n and A-n series, respectively. The corresponding percentage values of phosphorus in combination with other bases than iron were 84.4 and 76.5 for the N-n and A-n series, respectively, and indicate that 7.9 per cent. (84.4–76.5 = 7.5) more phosphorus was in combination with other bases than iron. The differences between the

TABLE IX

AMOUNTS OF IRON AND PHOSPHORUS AND RATIOS BETWEEN THESE AND ALSO BETWEEN THE THEORETICAL RATIO (1.77) IN THE ROOTS OF THE DIFFERENT CULTURES AND PERCENTAGE VALUES OF PHOSPHORUS IN COMBINATION WITH BASES OTHER THAN IRON

Cultures	Nitrate series					Ammonium series				
	Fe	P	Ratios		Px†	Fe	P	Ratios		Px†
			$\frac{Fe}{P}$	$\frac{O}{T}$ *				$\frac{Fe}{P}$	$\frac{O}{T}$ *	
	mg./gm.	*mg./gm.*			%	*mg./gm.*	*mg./gm.*			%
Plus iron	0.220	0.0790	0.273	0.1565	84.4	0.0282	0.0680	0.4150	0.2345	76.5
Minus iron	0.0012	0.0460	0.026	0.0147	98.5	0.0003	0.0380	0.0079	0.0045	99.5
O–F					14.5					23.0

* $\frac{O}{T}$: O = observed $\frac{Fe}{P}$ ratio; T = theoretical ration of $\frac{Fe}{P}$ = 1.77.

† Px = phosphorus unaccounted for, that is, in combination with other than Fe or a constituent of root cells; was obtained from $1.0 - \frac{O}{T} \times 100$.

O cultures were insignificant. The precipitation of greater amounts of phosphorus on the roots of the plants of the N-n than of the A-n series suggest that the higher pH values of the former cultures were responsible for the extra amounts of precipitated phosphorus. The data further suggest that the smaller amounts of phosphorus and iron in the leaf and stem sections of the plants of the N-n than of the A-n series of the F cultures were caused by the precipitation of both iron and phosphorus under the high pH conditions of the cultures of the former series. The results indicate, generally, that iron (and in certain cases phosphorus) deficiencies might develop in plants during the absorption of certain nutrients by roots from solutions favoring generation of high pH values and insolubleness of these elements.

Regarding the effects from the amounts of iron in the F and in the O cultures on the accumulation of various nutrient elements in plants, the statistical data in table VIII are of considerable interest. They show that the amounts of dry matter and iron increased while magnesium decreased significantly in the F cultures of both nitrogen series. The amounts of certain other elements were affected differently by iron. For instance, the accumulation of calcium was increased in the plants of the F cultures of the N-n series, but decreased in those of the A-n series. The opposite condition was found in the amounts of ash and potassium which were high in the plants of the O cultures of the N-n series and low in those of the A-n series.

Summary

1. Growth of *A. comosus* in minus-iron solution cultures (with traces of Fe from C.P. nutrient salts) was approximately as good as it was in plus-iron cultures when supplied with ammonium-nitrogen salts as a source of nitrogen. In solution cultures with nitrate-nitrogen salts, however, the growth in the minus-iron cultures was appreciably inferior to that in the plus-iron cultures.

2. Average drift of H-ion concentrations during the 2-week intervals between renewal of the nutrient solutions was from pH 6.6 to 4.2 for the ammonium-nitrogen series, and from pH 4.4 to 6.8 for the nitrate-nitrogen series. Precipitation of iron and decrease of its availability to plants was caused by rising pH values, as in the nitrate-nitrogen series. Iron availability was relatively high in the cultures of the ammonium-nitrogen series because of the lowering of pH values.

3. Iron was absorbed at exceedingly low rates from the solution cultures of the nitrate-nitrogen and ammonium-nitrogen series. Although the amounts of iron translocated from the roots to the leaves were greater in the plants of the ammonium-nitrogen than of the nitrate-nitrogen series, those precipitated on the roots were relatively high in both series.

4. Amounts of chlorophyll (to be reported in a subsequent publication), rather than of iron, were observed to constitute a better criterion for measuring the response of plants to different iron applications.

5. Phosphorus was found to increase slightly the amounts of iron precipitated on the roots of plants.

6. More manganese was found in the roots and less in the leaves of the plants of the plus-iron than of the minus-iron cultures, suggesting possible precipitation by iron.

7. The amounts of ash and particularly of potassium were higher on a percentage but not on a per plant basis in the plants of the minus-iron than of the plus-iron cultures of the nitrate-nitrogen series. In the ammonium-nitrogen series the differences were small and in some cases the results were reversed.

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FLAGELLATES OF LATICIFEROUS PLANTS[1]

R. B. Harvey and Sylvan B. Lee

(WITH FOURTEEN FIGURES)

Introduction

Since flagellates have been reported to be present in a large number of laticiferous plants, the increased interest in the possibility of using these plants for sources of rubber, and their introduction into the agriculture of new regions of the United States and other American countries has made desirable further studies on these organisms. After the statement was published by Hegner (**2**) that flagellates had not been found commonly in economic rubber plants, their occurrence in *Taraxacum kok-saghyz* has been reported by Isakova (**4**). A survey of the literature shows that they have been reported for a number of rubber plants in their native habitats. The introduction of such plants into extensive culture may make more frequent the usually sporadic occurrence of these flagellates, provided their insect vectors also become more prevalent in mass cultivation of the host plants used for rubber production. Without the presence of natural vectors, infestation may be present only in plants vegetatively propagated since the flagellates have not been shown to be transmitted through seed.

Some of the laticiferous plants have been suspected of being reservoirs for flagellates that produce animal and human infections causing tropical ulcers. Various skin ulcers of the Brazilian type of Leishmaniasis are said to be very common among persons who are engaged in clearing uncultivated woodlands, as in the clearing of rubber plantations (**7**). The transmission of plant flagellates to mammalian hosts, however, has been only occasionally successful. Strong (**9**) found flagellates morphologically identical in the latex of a Euphorbia, in a hemipterous insect feeding on this plant, and in a lizard feeding on the insects; he produced a skin ulcer in a monkey by injecting the intestinal contents of the lizard. Non-flagellate forms from the ulcer were morphologically similar to Leishmania.

Effects of flagellates on host plants

Some authors have reported symptoms of the hosts that indicated a pathological condition in flagellate infested plants. Holmes (**3**) has indicated that *Herpetomonas elmassiani* in the latex of *Asclepias syriaca*, produces little or no symptoms of pathogenicity. Heavy infestations make the latex somewhat watery. Wenyon (**10**) states that starch and other latex granules may disappear, causing a watery appearance of the latex. It is difficult to conclude that the presence of such large numbers of flagellates as occur in latices, can have no effect on the constituents of the latex. From experience in the transplanting of infected and healthy plants of the same

[1] Paper no. 50, Journal Series, Chemical Research Department, General Mills, Inc.

species, a weakness of the infected plants seems to result in decreased transplant survival. This was especially noticeable in Chamaesyce species in which the flagellates were present in great numbers. Also, infested plants did not survive well the removal of leaves and flower clusters for the purpose of examining and obtaining latex for culture of the flagellates in artificial media.

Occurrence of flagellates in laticiferous plants

The authors have taken as the criterion of positive infestation of a plant by flagellates, the identification of motile flagellates in microscopic amounts of fresh, undiluted, and uncontaminated latex and the confirmation by a second observer. If their motion was too rapid, or the latex too opaque to identify the presence of flagella in motion, a mount stained with Wright's blood stain was used to confirm the presence of a flagellum on the organism. Such stained mounts have been preserved to authenticate the identification. Living, infested plants were maintained for several months to facilitate study of variations of the flagellates and their effects on the host plants. Moderate dilution of latices with distilled water caused swelling and plasmoptysis within 10 to 30 seconds. Sterile normal salt solution (NaCl 0.8 per cent.) causes some swelling, but maintains motility of the flagellates for several hours. Mounts in normal saline solution should not be used for making dimensional measurements. The drying incident to the use of solvents in Wright's blood stain decreases the diameters of thick-bodied forms. The dimensions of body and flagellum are changed by longitudinal division. Latices in which many dividing flagellates are seen, generally show many thin forms.

Flagellates have been reported previously in 28 species of the Euphorbiaceae (**9**), 5 species of 4 genera of the Asclepiadaceae, 5 species of 4 genera of the Apocynaceae, 1 species of the Sapotaceae, and 3 species of Ficus of the Urticaceae. In the Compositae they have been reported in *Taraxacum kok-saghyz* (**4**). The reliability of a number of identifications has been questioned because cell inclusions, elongated nuclei, and protein aggregates, may have a flagellate-like appearance, especially in stained sections. Of the commercial rubber-producing plants, flagellates have been reported only in *Cryptostegia grandiflora, Funtumia elastica,* and *Taraxacum kok-saghyz.* At the U. S. Plant Introduction Garden, Coconut Grove, Florida, in the neighboring areas, and on the Keys, 12 species of plants were found that have not been reported as hosts of the flagellates.

Flagellates of somewhat different morphological characters and different host of origin were found in the families Euphorbiaceae and Asclepiadaceae; in the genera Poinsettia (SMALL's classification), Chamaesyce, and Funastrum; and in 14 species, as follows: *Poinsettia heterophylla* (L.) Small, *P. cyathophora* (Murr.) Small, *P. pinetorum* Small, *Chamaesyce adenoptera* Small, *C. buxifolia* (Lam.) Small, *C. deltoidea* (Engelm.) Small, *C. hirta* (L.) Millspaugh, *C. hypericifolia* (L.) Small, *C. hyssopifolia* (L.) Small,

C. maculata (L.) Small, *C. mathewsii* Small, *C. polygonifolia* (L.) Small, *C. tracyi* Small, and *Funastrum clausum* Jacq.

In the genus Poinsettia, flagellates had previously been reported in *P. heterophylla*. *P. pulcherrima*, the common ornamental shrub, showed no flagellates in the white, pink, red, or double red varieties. Hemipterous insects were seen travelling between *P. heterophylla* and *P. pulcherrima* and seem commonly to feed on both when in close proximity.

Of the genus Chamaesyce, all of the species found in a limited survey of the region of South Florida showed flagellates present in certain individuals. *C. hirta, C. hyssopifolia, C. hypericifolia, C. adenoptera, C. conferta, C. maculata,* and *C. mathewsii,* are extensively infested. *C. buxifolia* is usually infested, *C. tracyi* less commonly, and *C. deltoidea* rather rarely infested. The genus Chamaesyce is very large, and other regions will need to be searched to find if flagellates may be present in all the species of it.

Hundreds of specimens from other genera of laticiferous plants were examined without finding flagellates. These are reported as genera, and indicate that although flagellates were commonly found in the species here reported from the same locality, these genera did not become infested at the U. S. Plant Introduction Garden: Adenium, Alstonia, Asclepias (3 species), Calonyction, Castilla, Catharanthus, Convolvulus (3 species), Croton, Cryptostegia (3 species), Echites, Euphorbia (4 species), Ficus (20 species), Funtumia, Hevea, Ipomea (2 species), Landolphia, Manihot (2 species), Masconhazia, Metastelma, Musa (2 species), Pedilanthus, Phyllanthus, Rhabdadenia, Rhus (2 species), Ricinus, Stillingia (2 species), Strophanthus (2 species), Taraxacum (2 species), and Ursola.

Infected plants of the species examined growing in their natural habitats show no visible symptoms of disease, decreased vigor or green color. Infection can be determined only by microscopic examination for the flagellates. Watery latex cannot be used as a criterion of infection. Flagellates were found in very viscous, creamy latex of *Funastrum clausum*, growing in a dry soil. In the U. S. Plant Introduction Gardens of Coconut Grove, Florida, *Chamaesyce conferta* growing in cracks of bituminous pavement showed general infestation in dry weather; after a heavy rain, flagellates were hard to find. *Chamaesyce hypericifolia* growing in coral rock in full sunlight showed a high incidence of infestation whereas large vigorous plants growing in shade on moist glade land immediately adjacent showed no flagellates. *Poinsettia heterophylla* showed infestation of one branch tip of a plant 8 feet tall, but no flagellates in latex from another branch. A distance of 4 feet of stem separated the tips of these branches.

The flagellates frequently disappear from the latex of petioles of leaves and from the peduncles of flower clusters. After a rain of several hours, a potted plant (*P. cyathophora*) that in days previous thereto had shown numerous flagellates, failed to show their presence in any petiolar latex, whereas the flagellates remained in a plant whose leaves were protected from this rain.

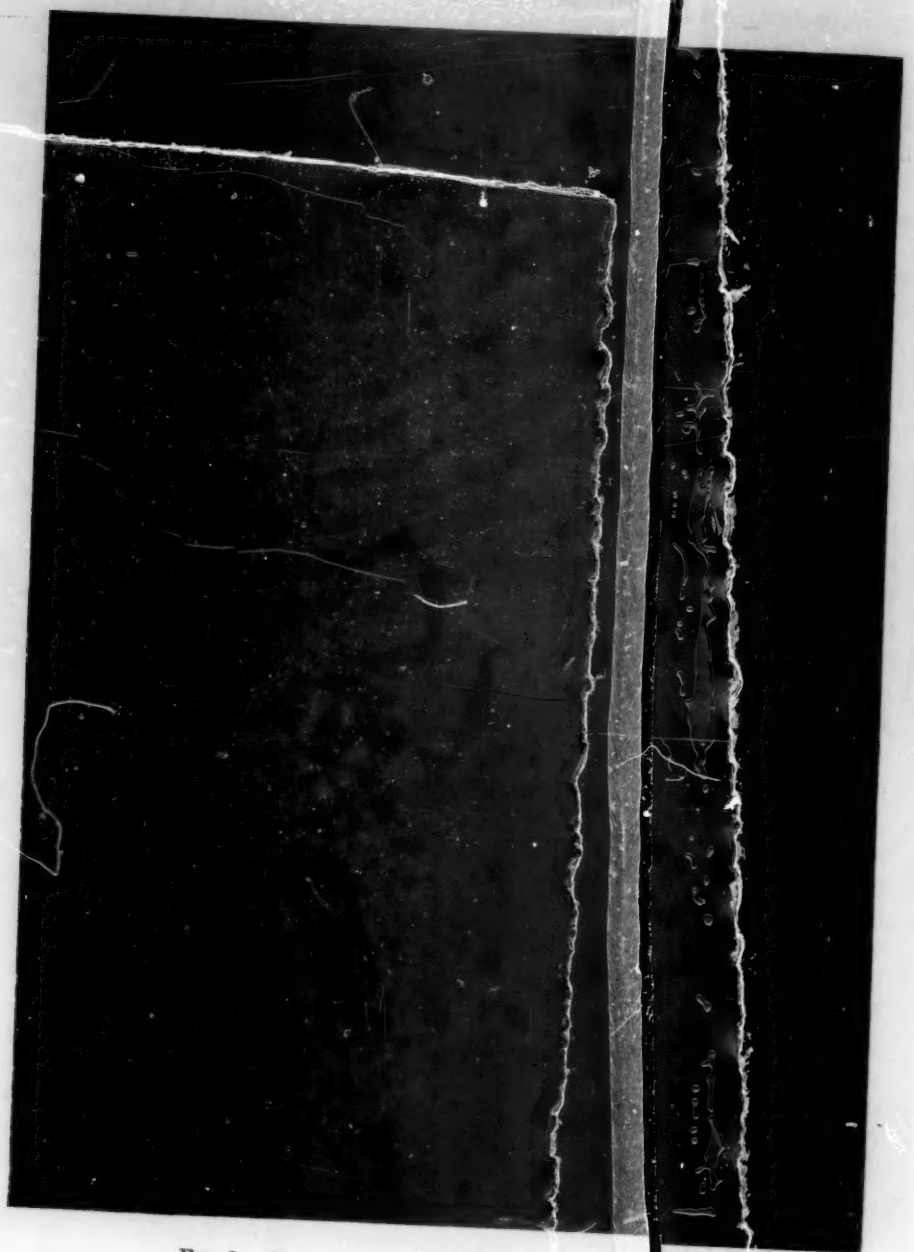

FIG. 1. *Chamaesyce adenoptera* flagellate (×12[illegible]0).

FIG. 2. *Chamaesyce buxifolia* flagellate [involution forms (×1220)].

FIG. 3. *Chamaesyce conferta* flagellate (×1220).

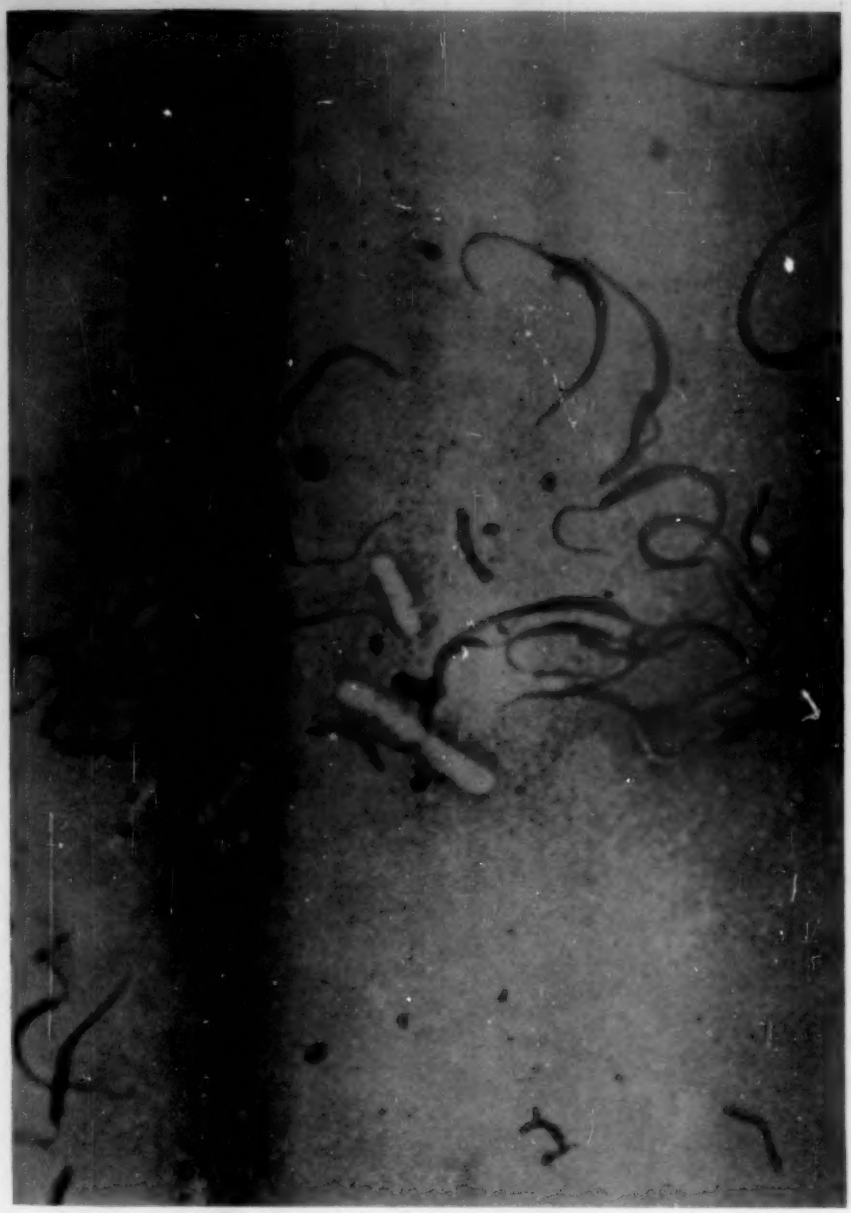

FIG. 4. *Chamaesyce deltoidea* flagellate (×1220).

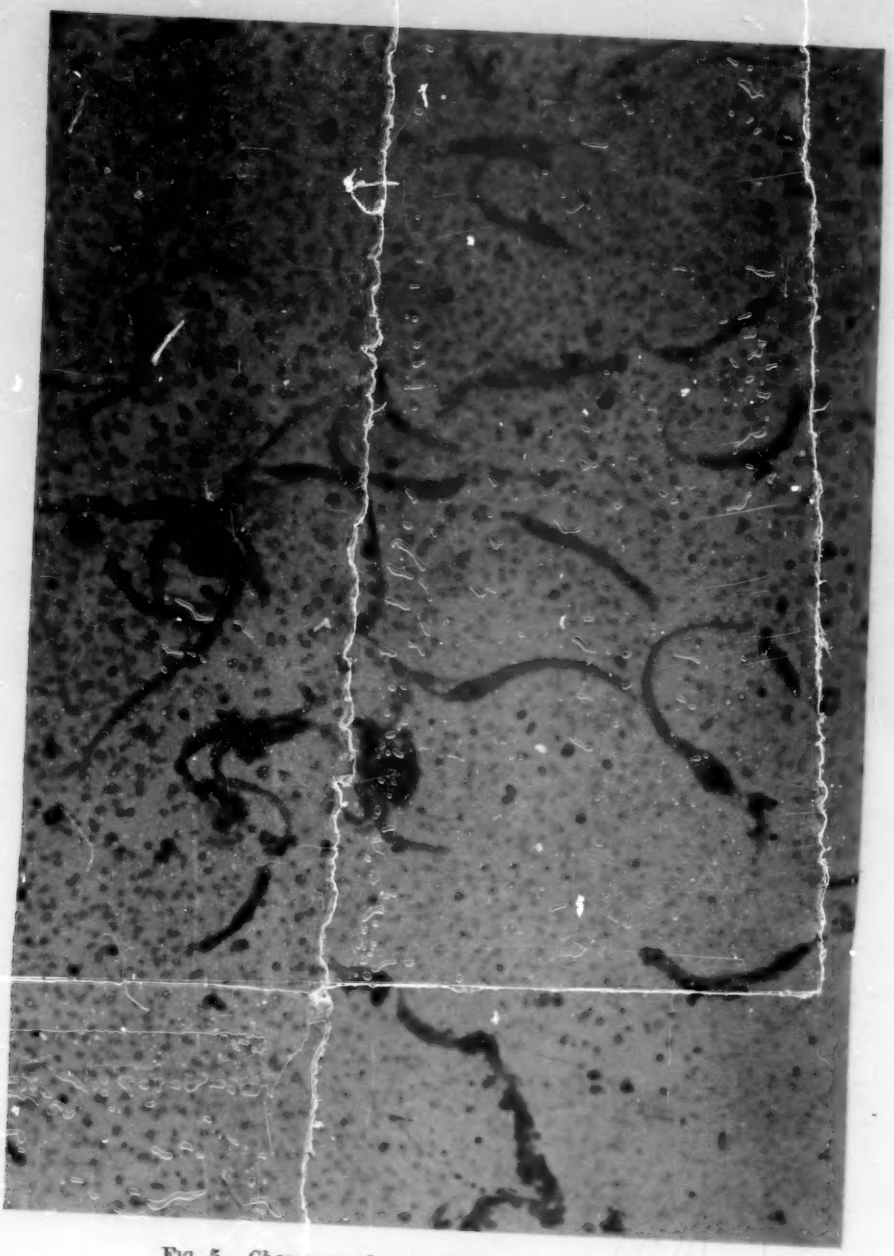

FIG. 5. *Chamaesyce hypericifolia* flagellate (×1220).

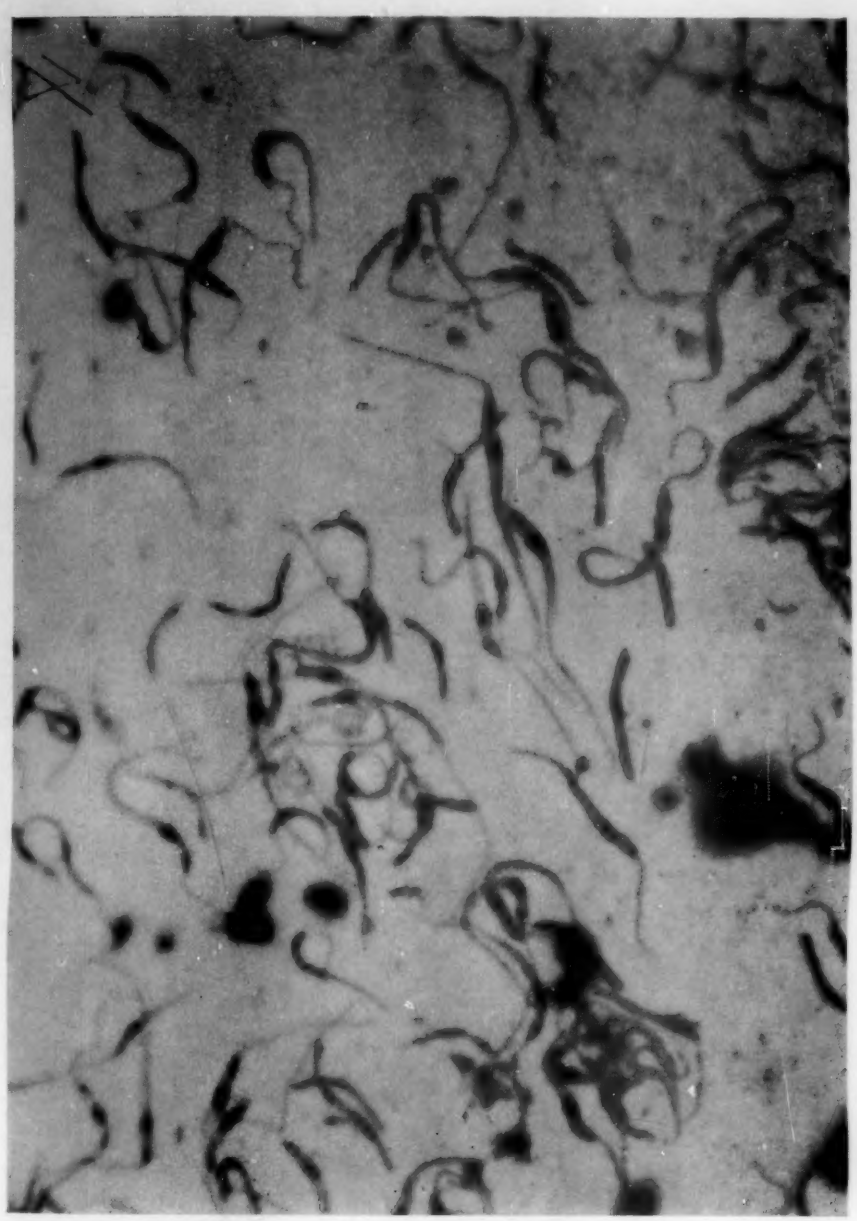

FIG. 6. *Chamaesyce hyssopifolia* flagellate (×1220).

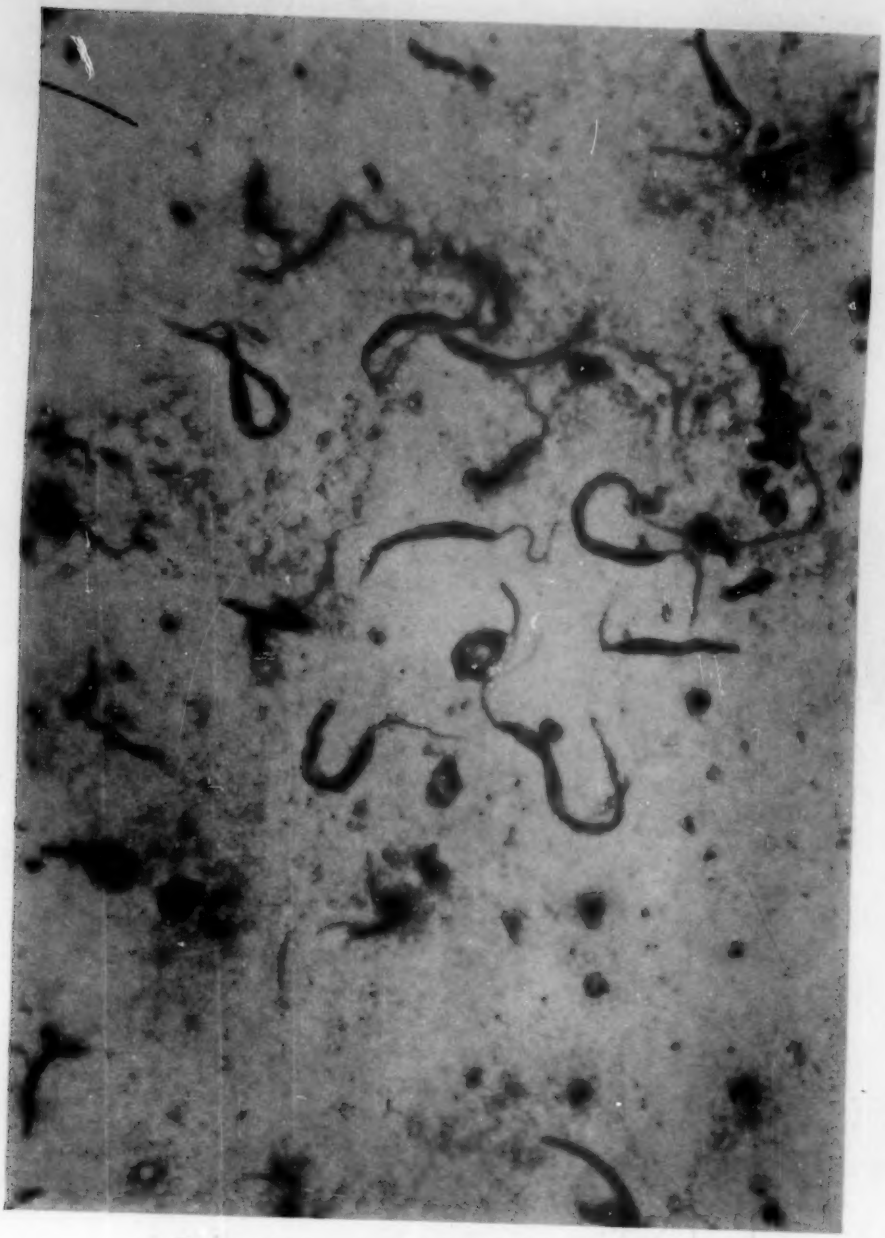

FIG. 7. *Chamaesyce maculata* flagellate (×1220).

FIG. 8. *Chamaesyce mathewsii* flagellate (×1220).

FIG. 9. *Chamaesyce polygonifolia* flagellate (×1220).

FIG. 10. *Chamaesyce tracyi* flagellate (×1220).

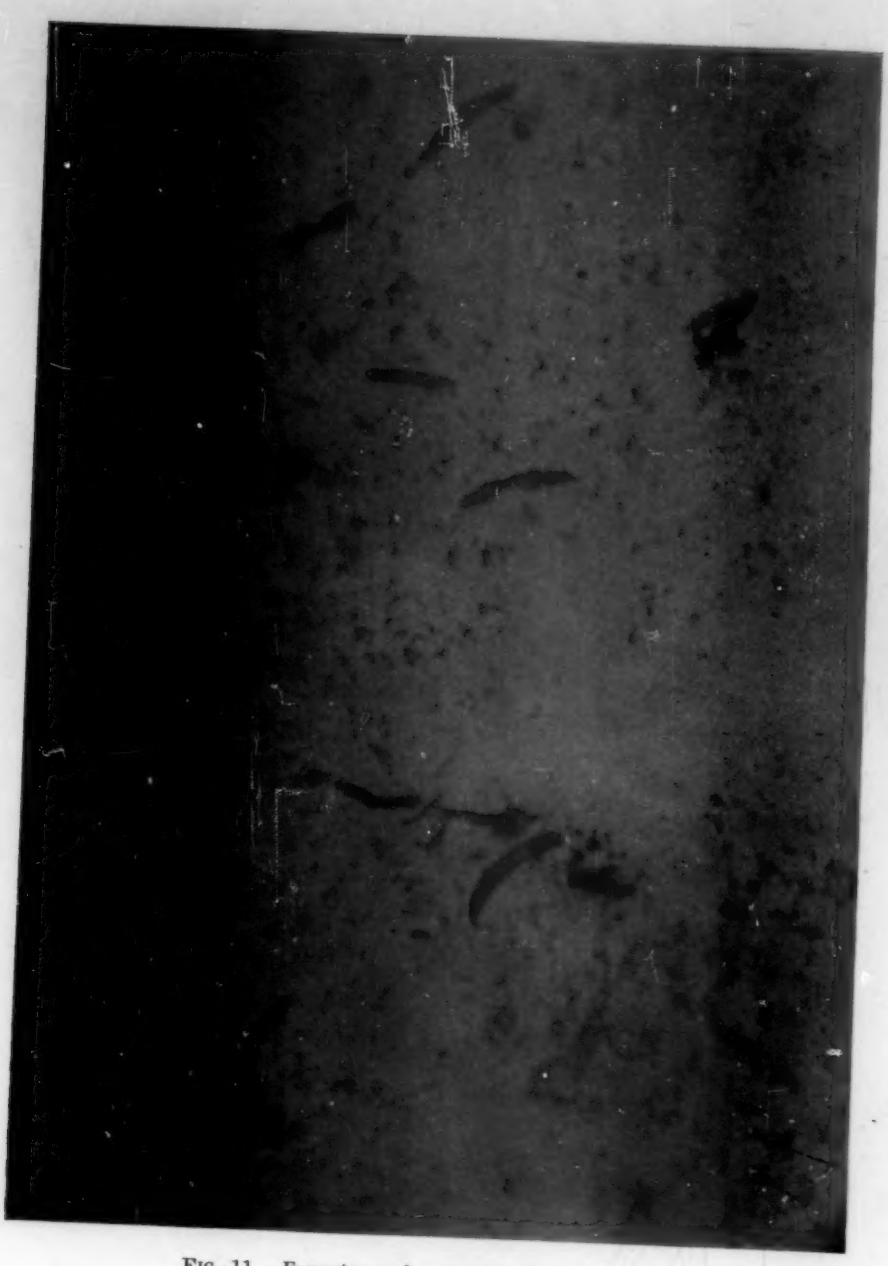

FIG. 11. *Funastrum clausum* flagellate (×1220).

FIG. 12. *Poinsettia cyathophora* flagellate (×1220).

FIG. 13. *Poinsettia heterophylla* flagellate (×1220).

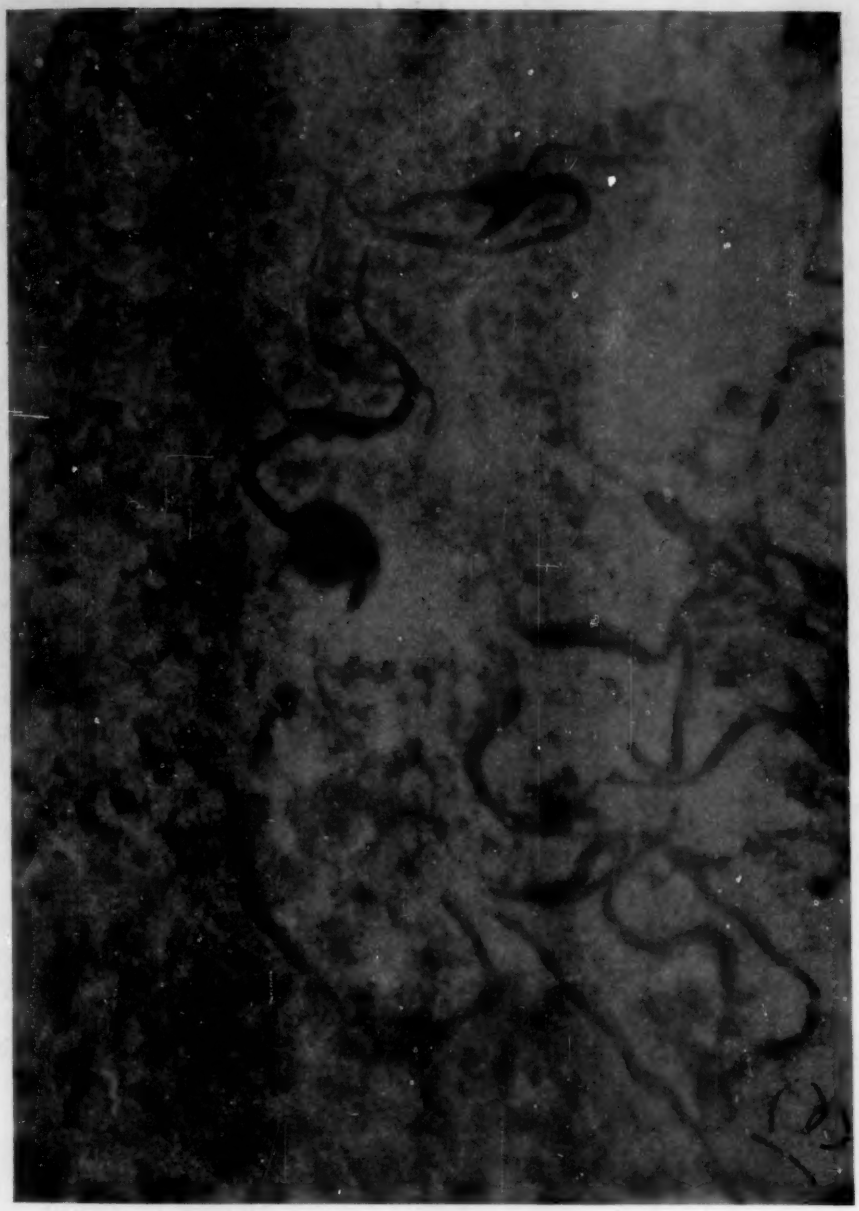

FIG. 14. *Poinsettia pinetorum* flagellate (×1220).

Flagellates are difficult to count for actual incidence (to obtain a picture of their normal distribution in a plant) on account of the mobility of the latex and also of the organisms themselves. Crude estimates on petiolar latex of *Poinsettia heterophylla* show greatest abundance in the red flag leaves at the tips of the branches, decreasing toward the base of the plant. In latex from the successive nodes of the stem, the greatest number was found in the latex of terminal nodes with decreasing numbers toward the base, then an apparent increase in numbers above the soil level.

Funastrum clausum, infested vines 50 to 70 feet long, showed abundant flagellates in the green tips but none in the brown bark covered stems above the point of rooting.

Culture of latex flagellates

Attempts have been made to study numerous latex flagellates in artificial culture. Upon reviewing previous work on these microorganisms, HOLMES (**2**) states: "Cultures of latex flagellates are difficult to establish. Suitable media may be inoculated with the organisms many times without the formation of successful colonies."

FRANCHINI (**1**) and NIESCHULZ (**6**) used plates of NÖLLER's horse blood agar in the cultivation of a flagellate from a plant of *E. cereiformis*. This medium is a nutrient broth containing agar and glucose (1 per cent.) to which an equal part of defibrinated horse blood is added before pouring plates.

MIGONE (**5**) used human blood agar to culture a member of the genus Herpetomonas from the host plant *Morenia odorata*. NOGUCHI (**8**) mentions the difficulty of adaptation of the flagellates to the media and obtained pure cultures of plant flagellates only on leptospira medium and only three times among many attempts. He obtained cultures of 2 species of flagellates from plant latices: *Hepetomonas oncopelti* from *Asclepias syriaca* and *A. nivea;* and *H. lygaeorum* from *A. syriaca*.

Culture of the flagellates of all of the genera was attempted on many hundreds of media modifications of those already successfully used for flagellates of animal diseases. Although survival of motile forms was seen for 4 to 5 days in the most favorable of these media, mass multiplication was obtained only in a rich medium injected into live coconuts containing their natural milk, and in the same medium in flasks to which Hevea latex was added.

For inoculation, petioles of leaves, peduncles of flower clusters, or tips of stems were first swabbed with Lugol's iodine solution, or tincture of iodine. This treatment did not kill the flagellates within the laticiferous system if the solvent was allowed to dry before cutting into the laticiferous tubes. On cutting the plant with a flamed scalpel or knife, the latex exuded in drops which were collected in a flamed platinum loop dipped into the sterile medium before touching the latex.

Transfer to normal salt solution was used in some cases to obtain a quantity of inoculum that later was transferred to the medium by loop or

into coconuts by sterile glass syringe. In inoculating coconuts the best procedure was to remove the husk and sterilize the largest of the embryo spots on the shell with tincture of iodine. A hot needle was then used to puncture the soft tissue of the coconut embryo. Injection of medium and inoculum from the syringe generally did not require removal of milk from the cavity in nearly ripe fruit. In green fruit the milk cavity usually exhibited a strong hydrostatic pressure, and some milk must be removed to allow additions. The removal of the embryo plug by a hot cork borer did not generally give preparations as free from contamination as did needle puncture. A wad of cotton containing iodine tincture was then placed over the inoculation point and covered with adhesive tape. In needle punctures, the endosperm swelled up, closing the puncture, and preventing drying. In the removal of embryo plugs there was more loss of moisture through the cotton plug and more contamination from fungi growing over the shell.

The best basic medium was:

Beef extract (Difco)	1.5 gm.
Peptone (Difco)	2.5 "
Yeast extract (Difco)	2.6 "
Glucose	3.3 "
Na acetate	3.3 "
Distilled H_2O	1000 ml.

Fresh rabbit's blood (5 per cent.) was added aseptically from the marginal vein of the ear. The final pH was 7.0.

The acidity of this medium was varied from pH 3.0 to 8.0. The osmotic pressure was varied by adding 0.0 to 0.9 per cent. sodium chloride and the cultures were incubated under aerobic and anaerobic conditions at temperatures ranging from 20° to 37° C. From a study of these conditions with the above medium it was found that aerobic conditions at pH 7.0 with no added sodium chloride and a temperature of 30° C. gave most favorable results. With this medium and these conditions flagellates were demonstrated alive and motile after periods of 9 to 12 days.

Attempts were made to improve this medium by the addition of sterile (Berkefeld filtered) preparations of the following growth factors with amino acids, and cyclic nitrogen bases at levels equal to or slightly higher than those found effective for other microorganisms: thiamine, riboflavin, pyridoxine, pantothenic acid, nicotinic acid, para-amino benzoic acid, biotin, folic acid (concentrate), inositol, glutamine, asparagine, tryptophane, glutamic acid, arginine, cystine, glycine, lysine, histidine, threonine, proline, alanine, valine, hydroxyproline, methionine, β-alanine, leucine, cysteine, adenine, guanine, uracil, and xanthine.

Modifications of the above media, as well as several other media containing blood, have been tested for their ability to support the growth of latex flagellates. Also rabbit hemoglobin, fresh and pasteurized rabbit

serum, and beef blood were used to replace the rabbit blood in the above medium, but without good growth.

Egg media

Fertile and non-fertile eggs were inoculated directly into the yolk and albumen with latex flagellates. The results were negative after incubation periods of a few days to several weeks. Several other types of egg media were tested. These include: modified Boeck's Locke's medium, Hogue's egg medium, and Schneider's and Boeck's Locke's egg medium plus blood serum. The results were negative.

Difco media

Numerous media prepared by Difco for the culture of fastidious microorganisms were tested for their ability to support the growth of latex flagellates. These included: veal infusion, brain-liver-heart, brain-heart, cabbage infusion, blood-base-agar plus rabbit blood, wort agar, egg-meat broth, and potato infusion. These media were tested alone and in combination with rabbit blood and the growth factors, amino acids, and nitrogen bases listed above. Results were negative.

Coconut culture

Coconut milk has been used by previous workers in attempts to culture flagellates. Injection of media with flagellates into live coconuts has worked with some success. The coconuts were prepared as described above. The media and the inoculum were injected into the coconuts through the sterilized embryo spots. The pH inside the coconuts was raised from 5.0–5.5 to 7.2 by injecting sterile M/1 K_2HPO_4, and sterile supplements of various types were added. The inoculum consisted of a suspension of latex organisms in sterile saline (0.8 per cent.). Using this technique it was possible to demonstrate the presence of numerous motile flagellates after 30 days in a coconut which had been fortified with the rabbit blood medium listed above. Sub-cultures from these coconuts failed. To other coconuts adjusted to pH 7.2 with phosphate buffer, the mixture of growth factors, amino acids, and nitrogen bases listed above was added. No growth was obtained in these media.

Culture in Hevea latex

In the above basic medium with fresh unsterile Hevea latex added, cultures of flagellates from *Funastrum clausum* with rapid multiplication were obtained. The flagellates grew more rapidly than the contaminants at 37° C. and after 12 hours were very numerous and actively motile. The flagellates from *Funastrum clausum* grew and divided into single individuals and also formed long chains of 20 to 30 flagellates that moved with a spiral motion. These chains were easily broken apart by shaking the flask or in making mounts. After 24 hours the flagellates had become immobile or disappeared and the medium was heavily contaminated by motile and

non-motile bacteria, spirillum, and coccus forms. Subculture after 12 hours was attempted in the same basic medium to which was added the serum obtained by precipitating the rubber from fresh Hevea latex with the least amount of acetic acid to obtain a serum separation, neutralization of the serum with NaOH, and passing through a Berkefeld filter. No flagellates grew but contaminants were numerous. Inoculation of a similar sterilized preparation with Hevea latex serum with fresh inoculum from Funastrum gave no growth of flagellates.

In examining latices for fluorescence, an all quartz illumination system was used with light from the G.E. AH4 100-watt mercury arc in clear Corex glass. Flagellates exposed on a quartz slide to the intense ultraviolet and visible light transmitted by this system were very active after 4 hours. Hence the organism seems insensitive to ultraviolet and visible light. Attempts to grow the flagellates in pyrex flasks in sunlight using several nutrient media were not successful.

It is apparent from these cultures that the latex flagellates are difficult to culture and require essentially media derived from live tissues. To obtain such in a sterile condition one is limited to the use of coconuts since it is practically impossible to obtain other sterile plant latices in quantity. NOGUCHI (**6**) has reported that the flagellates of *Euphorbia braziliensis* and *Asclepias curassavica* will not grow in sterile coconut milk media *in vitro*. This is confirmed. A number of reports of culture of these organisms seem to be questionable. Perhaps survival in the media for a few days rather than actively dividing cultures were obtained.

Descriptions

All the photographs (figs. 1 to 14) are at the same magnification (×1220) to facilitate comparisons of flagellate dimensions. The latex flagellates in Poinsettia, Chamaesyce, and Funastrum have a single anterior flagellum, arising anterior to the parabasal body. In Funastrum, the flagellum is very short. In the photographs these are not easily recognized, but in live organisms they can be seen in motion. The flagella in Poinsettia and Chamaesyce are frequently longer than the body. The characteristic movement of the flagellum can be seen in the long ones to be a pulsating, spiralling coil motion from the body passing toward the end. Frequently more than one coil can be seen travelling out on the flagellum, as in a rope that is given quick circular movements by the end held in the hand.

The blepharoplast and nucleus differ in their size, position, and density of staining in the different species of hosts. Some nuclei are large rounded and distinct (*C. buxifolia*, *C. hypericifolia*, *C. hyssopifolia*, *C. mathewsii*, and *C. tracyi*) others are elongated and have indistinct outlines (*C. adenoptera*, *C. deltoidea*, and *C. polygonifolia*).

Twisted ribbon-like bodies are commonly seen, and the motion of the body is in a spiral path through the latex. In chains of flagellates, the movement of the whole series of flagellates is coordinated, giving a ser-

pentine-like movement and rolling the chain. This movement also is characteristic in the twisted ribbon-like forms. In Funastrum only thin twisted ribbon-like forms with blunt-ended bodies were seen. In other latices these same forms occurred: *C. buxifolia, C. conferta, C. deltoidea, C. hirta, C. mathewsii, C. polygonifolia, C. tracyi;* but less commonly in the Poinsettia species. The commonly long flagellated rounded bodies that taper posteriorly to a tail sometimes of a diameter as small as the flagellum, are seen in all of the species of Chamaesyce and Poinsettia. The method of longitudinal division of the body can be seen in the photograph of *P. cyathophora* latex, where two blepharoplasts, two nuclei, and one flagellum can be seen in the flagellate with the rounded body at the center of the figure. More than two bodies can frequently be seen united at the posterior end in rapidly dividing cultures or latices. In *Chamaesyce buxifolia,* in addition to the flat twisted ribbons and rounded tapering bodies, spherical and distorted bodies are commonly found in active motion by their flagella, or quiescent without flagella. These forms may be produced by abnormal water absorption from rain water or tap water used to water the plants, because this species commonly grows in habits of high salinity near the sea coast. In cultures from *C. buxifolia,* the ribbon like forms with 45 degree spiral turn have blunt ends and survive longer than the other forms.

Summary

Twelve species of plants are newly reported as hosts of latex flagellates. Photographs of the flagellates from different species are given in the text.

The incidence of infestation was variable in Chamaesyce species, but individuals were found infested in all of the species of this genus that were examined.

Incidence of infestation was found variable with habitat, soil, and weather conditions.

Culture for 30 days was obtained in a medium injected into live coconuts and rapid multiplication occurred in a similar medium to which unsterile fresh Hevea latex was added.

The authors are obliged to Dr. Russel Seibert, Rubber Plant Investigations, U. S. D. A., for determinations of species.

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COMPOSITION OF THE ROOTS AND STUBBLE OF PERENNIAL RYEGRASS FOLLOWING PARTIAL DEFOLIATION[1]

J. T. Sullivan and V. G. Sprague

(WITH NINE FIGURES)

When the foliar portion of a grass plant is removed by a mower or by a grazing animal, the growth of new tissues is initiated to replace the parts removed. The rate with which the regeneration of new top growth proceeds and the total amount produced are of great importance in forage production. This rate of recovery and total yield, although dependent to a large extent upon external environmental factors, are also influenced considerably by the intensity and frequency of defoliation as determined by management practices. Evidence has been offered by many workers (**5, 9, 10, 11, 21, 23, 24, 26, 29**) that close and frequent clipping or grazing reduces the total yield of tops of forage plants.

Associated with the ability of the plant to produce new growth after cutting is its chemical composition. Plants cut less frequently and less severely have a greater opportunity to synthesize and maintain reserve food materials which are available for the production of new growth than plants more severely grazed. That carbohydrates are important as reserve substances in pasture plant economy has been pointed out by earlier workers (**1, 4, 7, 8, 13, 14, 15, 16, 25, 27, 28**).

The specific carbohydrates which may be considered "reserves" that can be utilized by the plants for purposes of respiration and production of new tissues have not been definitely established. Weinmann (**27**) considered that sugars and polysaccharides hydrolyzable by hot dilute HCl were lost from the tops of some South African grasses after the time of flowering and were largely recovered in the roots. In another contribution (**28**), the seasonal changes in the composition of roots were described as mainly due to translocation and storage. Sugars and starch (polysaccharides rendered soluble by saliva and hydrolyzed by HCl) were considered of importance but the results with total hydrolyzable polysaccharides were not conclusive and were not reported. In alfalfa, Albert (**2**), Graber, *et al.* (**8**), and Leukel (**13**) considered sugars, dextrins, starch, and hemicelluloses as available carbohydrates and found that the supply of these in the roots was lowered by frequent cutting of the immature tops. Plants in darkness (**2, 8**) produced new top growth while starch disappeared from the roots; total carbohydrates decreased and hemicelluloses increased. According to Bukey and Weaver (**4**) there was a marked decrease in the percentage of invert sugar, water-soluble hydrolyzable, and water-insoluble hydrolyzable

[1] Contribution no. 48, of the U. S. Regional Pasture Research Laboratory, Division of Forage Crops and Diseases, Bureau of Plant Industry, Soils and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture, State College, Pennsylvania, in cooperation with the northeastern states.

material in the underground parts of Andropogon under conditions of severe clipping. McCarty (**14, 15**) studied the seasonal fluctuations of sugars, starch, and hemicellulose in several range grasses during the growing season. Carbohydrate storage was inversely related to the rate of vegetative growth of the herbage. He concluded (**15**) that sugars and starches (chiefly sucrose and starch) were the more important of the stored carbohydrate foods. The behavior of the acid-hydrolyzable hemicellulose suggested that this carbohydrate was employed largely as structural material. Sugars and starch were also found by McCarty and Price (**16**) to be reduced in quantity in the roots of mountain brome and slender wheat grass if the plants were grazed during the reproductive period and during the normal storage period. Morozov (**17**) concluded from a study of brome grass, ryegrass, and fescue that the places of carbohydrate accumulation were the roots, rhizomes, stems, shortened internodes, and leaves. The carbohydrates stored were chiefly inulin-like substances and hemicellulose.

This inulin-like substance, resembling inulin only in that it yields fructose on hydrolysis, is probably the fructosan widely reported in recent years in the grasses and cereals. Norman (**18**) has reported its presence in ryegrass (Western Wolths) in amounts as high as 34 per cent. Norman and Richardson (**19**) report that it accumulates chiefly in the stem and acts as a temporary reserve, later being utilized for the production of structural constituents. The wide distribution of fructosan in pasture grasses (**3**) and the lack of confirmatory evidence of considerable amounts of starch make it appear probable that fructosan, rather than starch, is an important carbohydrate reserve. The properties of fructosan are such that in much quantitative work it may have been determined and reported incorrectly as starch. It is water-soluble and alcohol-insoluble, and is readily hydrolyzed to fructose by acids. When starch is determined by saliva or other enzymatic reagents in water solution fructosan may go into solution and be hydrolyzed by the subsequent acid treatment that often follows diastatic action. "Dextrin" and "starch" determinations reported in grasses may therefore have been largely of fructosan. This does not apply to such crops as alfalfa in which starch does occur and in which fructosan has not been shown to be present.

Carbohydrates readily change from one form to another so that by ordinary methods of analysis it is impossible to trace the translocation and utilization of any particular one in the plant. As a first step in the study of the relation of carbohydrates to regeneration of growth in grasses it seems desirable to have a knowledge of the carbohydrate changes taking place in the plant immediately after cutting. In this paper are reported the analyses of perennial ryegrass (*Lolium perenne* L.) plants at intervals after partial defoliation.

Materials and methods

The plant material used in this experiment consisted of a single clone of perennial ryegrass. In November, 1940, cuttings were made and planted in

gravel in one-gallon glazed pots, three tillers per pot, and supplied with a complete nutrient solution by automatic irrigation. The temperature of the greenhouse was maintained at 18 to 20° C. and the normal daylight period was increased to 14 hours with mazda lights at an intensity of approximately 75 foot candles. The average intensity of daylight in the greenhouse was 5,000 to 7,000 foot candles. Three replications were grown on three adjoining benches in the same greenhouse; each bench held one complete replication. In order to obtain sufficient material for analysis, three pots in each replication were composited at the time of harvest.

On March 17, 1941, about three months after establishment, the plants were vegetatively vigorous and had reached an average height of 8 inches. At this time the tops were cut with shears at a height of 1.5 inches above the surface of the gravel. The removed tops were dried and the dry weights determined. They averaged 5.40 grams per pot. They were not analyzed chemically.

The remaining stubble and roots were sampled for chemical analysis immediately after cutting. The plants from 3 pots selected at random from each replication were combined and, after washing and removing the gravel, the roots and stubble were separated. These samples were surface dried with paper towels and dropped into boiling alcohol. Later they were partly extracted, dried, coarsely ground, and completely extracted with 80 per cent. alcohol in the usual manner.

Sampling of the roots and stubble was repeated in the same manner 1, 2, 4, 7, 11, 16, 22, 28, and 36 days after cutting. At each date the new leaf growth which had appeared above the 1.5-inch level was removed and its dry weight only was determined. New leaf tissue below the 1.5-inch level was included in the stubble.

Another series of pots was removed to a darkroom immediately after cutting and plants from them were sampled in the same way and on the same subsequent days as were the plants allowed to recover in the greenhouse. Three pots were used to furnish a composite sample at each date for the material in the darkroom but no replication was available.

The alcoholic extracts were made to volume and an aliquot of each was used to determine total solids. The sum of the total solids of the extract and the alcohol-insoluble residue gave the total dry weight of the sample. Another aliquot of the extract, after removal of the alcohol and after clarification, was used for the determination of sugars. Reducing power was determined by a method essentially that of PHILLIPS (**22**). Fructose was determined by oxidizing aldoses with iodine by a procedure similar to that of NORMAN, WILSIE, and GAESSLER (**20**) and determining the residual reducing power. Sucrose was determined by hydrolyzing with hydrochloric acid and determining the increased reducing power. No other carbohydrates were believed to be present in the alcoholic extract. A portion of the alcoholic extract was used for a qualitative test for nitrates and the remainder was used for the determination of total nitrogen including nitrates. Nitrates were found only in plants growing in darkness.

The alcohol-insoluble residue was weighed and further ground to pass a 60-mesh sieve. Aliquots were used for the following determinations:

An extract of the residue was obtained by heating a 1-gram portion with 0.25 per cent. oxalic acid for 1 hour at 80° C., neutralizing with barium hydroxide, filtering, and clearing. Total reducing power and fructose were determined. The fructose was considered as having been derived from fructosan and is so reported, but calculated as fructose.

Cellulose was determined on another 2-gram portion by a modification of the method of CRAMPTON and MAYNARD (**6**).

A 1-gram portion was treated with normal sulphuric acid in a boiling water bath for 2.5 hours. The residue was removed and washed. The acid liquor and washings were neutralized with $CaCO_3$ and $Ba(OH)_2$, filtered, clarified, and made to volume. Determinations were made on aliquot portions for total reducing power, fructose, and non-fermentable reducing substances. The length of time of the hydrolysis, 2.5 hours, was sufficient to give the maximum yield of non-fermentable reducing power under the conditions of acid concentration and temperature. The non-fermentable reducing power was determined as the residual reducing power after treatment with bakers' yeast. It is expressed as pentosan (calculated as xylose) and the amounts agree closely with the furfural distillation method. Galactose could not be detected qualitatively. The total reducing power of the acid hydrolyzate, after correction for fructose (part of which had been decomposed during the acid treatment) was slightly higher than that of the pentose. The difference, amounting to a low percentage of the sample, was not further characterized. Part of it may be due to theoretical difficulties arising in the calculations of sugar mixtures.

The residue from the normal sulphuric acid treatment was treated with 72 per cent. sulphuric acid for 16 hours at 4° C. and finally with normal sulphuric acid for 5 hours at 100° C. The insoluble residue, after correcting for ash content, was characterized as lignin.

A portion of the alcohol-insoluble residue was used for the determination of total nitrogen by the Kjeldahl method.

Results

INITIAL COMPOSITION OF STUBBLE AND ROOTS

The analysis of variance of the dry weights of tops harvested at the beginning of the experiment (table I) showed that the differences among the

TABLE I

THE ANALYSIS OF VARIANCE OF THE DRY WEIGHTS OF THE TOPS REMOVED FROM THE PLANTS AT THE BEGINNING OF THE EXPERIMENT

VARIATION DUE TO	D/F	MEAN SQUARE	F VALUE
Dates	9	0.3193	1.12
Replications	3	0.9559	3.34
Dates × replications	27	0.2860	----

groups of three pots selected at each of the 10 dates of sampling were not statistically significant. It may be concluded that differential establishment of the plants was not an important factor in conditioning differences obtained at subsequent sampling dates.

The yield and composition of ryegrass stubble and roots, as determined on samples taken immediately after removing the tops, are given in table II.

TABLE II

THE YIELD AND COMPOSITION OF STUBBLE AND ROOTS OF RYEGRASS AT THE TIME OF DEFOLIATION ON MARCH 17, 1941. (EXPRESSED AS PERCENTAGE OF DRY WEIGHT, EXCEPT FIRST AND LAST ITEMS)

ANALYSES	STUBBLE				ROOTS			
	REPLICATIONS				REPLICATIONS			
	1	2	3	AVE.	1	2	3	AVE.
Total yield in dry matter, in grams per pot*	3.82	4.27	4.17	4.09	6.14	7.26	6.93	6.78
Total constituents accounted for	80.87	80.32	79.51	80.23	78.88	79.57	79.08	79.18
Dry matter soluble in alcohol	23.31	18.32	21.84	21.16	13.26	14.06	13.27	13.53
Fructose	1.93	1.62	1.81	1.79	0.85	0.90	0.89	0.88
Glucose	2.26	1.76	2.00	2.01	0.26	0.27	0.34	0.29
Sucrose	6.37	5.25	6.62	6.08	2.87	2.95	2.85	2.89
Fructosan	14.61	16.17	14.33	15.04	1.92	1.88	1.64	1.81
Aldose obtained from oxalic acid hydrolysis, as glucose	0.80	0.81	0.64	0.75	0.37	0.27	0.38	0.34
Pentosan, as xylose	15.71	16.65	16.27	16.21	23.54	24.02	24.04	24.53
Fermentable aldoses from sulphuric acid hydrolysis, as glucose	4.46	4.28	4.30	4.35	2.02	2.22	1.81	2.02
Cellulose	27.10	28.00	27.02	27.37	32.09	32.06	31.84	32.00
Lignin	3.98	3.95	4.03	3.99	12.04	12.25	12.41	12.23
Alcohol-soluble N	0.24	0.16	0.20	0.20	0.09	0.08	0.08	0.08
Alcohol-insoluble N	1.09	0.91	0.91	0.97	0.96	0.93	0.96	0.95
Total N	1.32	1.07	1.11	1.17	1.04	1.01	1.04	1.03
Alcohol-soluble N, in percentage of total N	17.75	14.70	17.99	16.81	8.42	8.09	7.94	8.15

* The average dry weights per pot of the tops removed on March 17 were 4.95, 5.09, and 4.89 grams, respectively.

The dry weight of the stubble and its composition depend upon the amount and the structures of the plant included in the tissues below the 1.5-inch level. In this case of vegetatively growing plants, they consisted primarily of leaf sheaths, a small amount of leaf blade tissue, and short stems which probably did not exceed ¼ inch in length. The yield of stubble in dry matter was a little more than half that of the roots. The stubble had greater concentrations than the roots of the more soluble constituents such as sugars, fructosan, and soluble nitrogen and less of the more insoluble constituents such as pentosan, cellulose, and lignin. About 21 per cent. of the total dry matter of the stubble was alcohol-soluble; if fructosan may be included, at least 30 per cent. was water-soluble. At least 15 per cent. of the roots was

water-soluble. Of the sugars, sucrose was considerably higher than either glucose or fructose in both the roots and stubble and was higher than fructosan in the roots. Of the two monosaccharides, glucose was the higher in the stubble and fructose in the roots. Fructosan was much higher in the stubble on a percentage basis and also on an actual basis, even though the total dry weight of stubble was less than that of the roots. The total constituents determined, using the factor 6.25 to convert nitrogen to protein, was about 80 per cent. of the dry weight of the stubble and 79 per cent. of the roots. Corrections for the contamination of lignin and cellulose with protein would have decreased these figures slightly.

Changes in dry matter after clipping

Dry matter changes of the different plant parts during the days following clipping are illustrated in figure 1. The yields reported, and also all

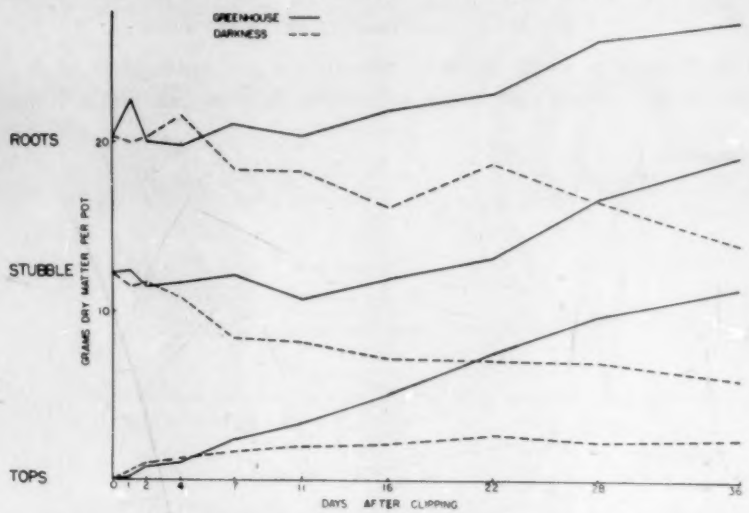

Fig. 1. Yield of roots, stubble, and new top growth of ryegrass plants growing in the greenhouse (solid lines) and in darkness (dotted lines) for 36 days after removal of the tops.

subsequent composition data, are the averages of three replications in the case of greenhouse plants and are of one replication in plants moved to the darkroom. A small but measurable quantity of new top growth above the 1.5-inch level was present on the first day after clipping. The rate of increase of the tops was fairly constant and the dry matter per pot amounted to 11 grams at the end of 36 days. In the dark, the rate of growth was similar to that of plants in light for about 5 days, after which it decreased. Total dry matter of regenerated leaves reached a maximum after about 22 days. During recovery in the greenhouse the stubble growth, including new leaf tissue below the 1.5-inch level, fluctuated somewhat, showed significant losses in total dry matter at several dates, and did not show a definite in-

crease above its original weight until after the 16th day. After 36 days of recovery there had been a net increase in weight of roots, stubble, and leaves amounting to 19 grams per pot. In darkness the loss of dry matter from the stubble was immediate. The roots, amounting to 20 grams of dry matter per pot, did not lose weight in the greenhouse and began increasing in weight after the 11th day, reaching 27 grams after 36 days. The roots in the dark room continually decreased in weight with a minimum of 14 grams on the 36th day after clipping. At the end of this period many of the plants in darkness were undoubtedly dead, though no attempt was made to revive them. Many roots were in stages of decomposition. Dry weight fluctuations in the roots and stubble during the first few days after clipping were probably due to pot variation. As an indication of pot variation the nine pots selected for the first group of samples had averaged 4.93 grams of top growth removed, as compared with total average for all pots of 5.40 grams.

Changes in composition after clipping

The changes in composition of the stubble and roots after clipping are illustrated in figure 2 and in all succeeding figures. In figure 2 are illus-

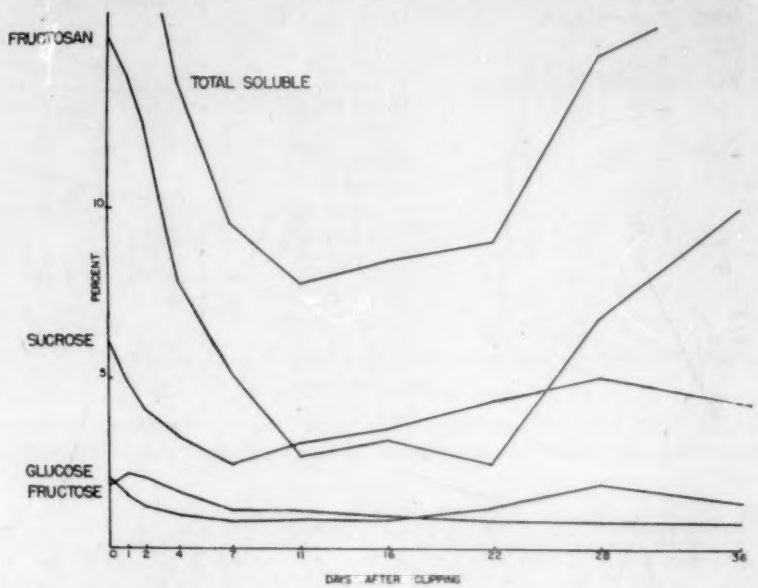

Fig. 2. Water-soluble carbohydrates of the stubble of ryegrass plants growing in the greenhouse for 36 days after removal of the tops.

trated the changes in the water-soluble carbohydrates, fructose, glucose, sucrose, and fructosan and also the total of these constituents in the stubble of plants growing in light from the day of clipping to 36 days thereafter. A rapid decrease in the percentages of all these constituents on the dry weight basis characterized the changes during the first seven days. Since no increase in dry weight had taken place in the stubble or roots during this

time it is assumed that these carbohydrate constituents were being translocated to the growing tops and utilized in part as raw material for new tissue and were also in part being decomposed by respiratory processes to furnish energy for such growth. The temporary increase of fructose on the first day may have been caused by the rapid hydrolysis of fructosan, which because of its high molecular weight (**12**) is not translocated as such. A minimum content of sucrose was reached about the 7th day and of other constituents soon thereafter. A rapid rise in all constituents occurred at a later date. The photosynthetic area of new top growth increased sufficiently so that sometime after 11 days of recovery re-storage of carbohydrates occurred. Whether re-storage was in the old tissue or new tissue could not be determined with the technique used since new as well as old leaf tissue was included in the stubble zone.

Figure 3 (on the same scale as fig. 2) illustrates similar changes in the roots. The concentrations in the roots were smaller, but the relative changes were as great.

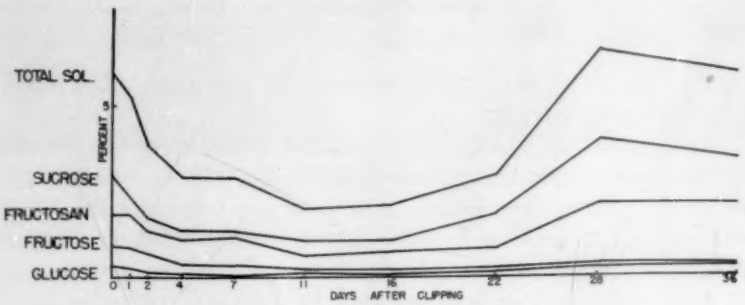

FIG. 3. Water-soluble carbohydrates of the roots of ryegrass plants growing in the greenhouse for 36 days after removal of the tops.

Figure 4 presents the changes taking place in the stubble with respect to the insoluble carbohydrates and lignin. Total soluble carbohydrates, represented by the dotted line, is repeated from figure 2 for comparison purposes. Cellulose, hydrolyzable pentosan, and lignin percentages did not show the typical U-shaped curve of the soluble carbohydrates. The apparent increase of these constituents during the first week may have been a real increase or may have been partly due to the method of expression in percentage of the dry weight. If soluble carbohydrates alone had been removed during this period all remaining constituents would have been increased on a percentage basis. Actual increase in total cellulose and pentosan may have occurred since some new tissue was formed. There was no evidence, however, of their utilization as in the case of soluble carbohydrates. After attaining a maximum at about 11 days there was a slight decrease in cellulose and pentosan. The basis of expression may again have been the cause of this since soluble carbohydrates have increased during this later period. Lignin did not show the later drop.

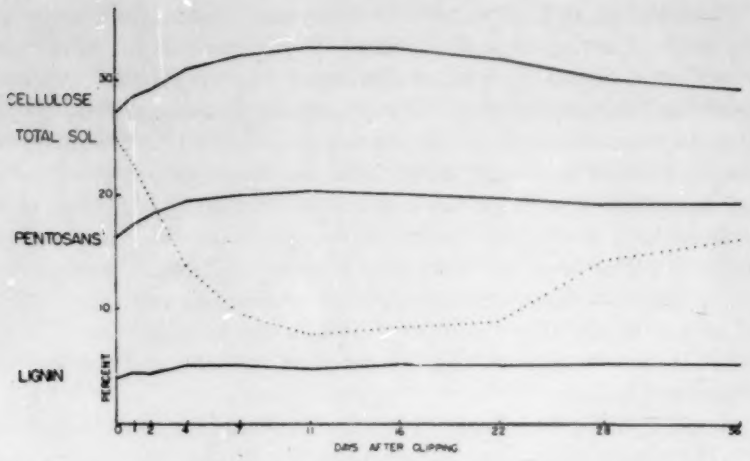

FIG. 4. Cellulose, hydrolyzable pentosans, and lignin (solid lines) and total soluble carbohydrates (dotted line) of stubble of ryegrass plants growing in the greenhouse 36 days after removal of the tops.

Figure 5 furnishes the same information for the roots. The conclusions to be drawn are similar.

Plants in darkness (figs. 6, 7) showed changes similar to those in the light during the period when top growth was being produced. Rates of loss of soluble carbohydrates were similar to those of plants in the light except that no re-storage occurred. All soluble constituents, if illustrated separately, would show similar changes. Fructosan disappeared entirely by the 22nd day in the stubble and by the 16th day in the roots. The soluble carbohydrates persisting to the 36th day were, in the stubble, 0.22 per cent. of

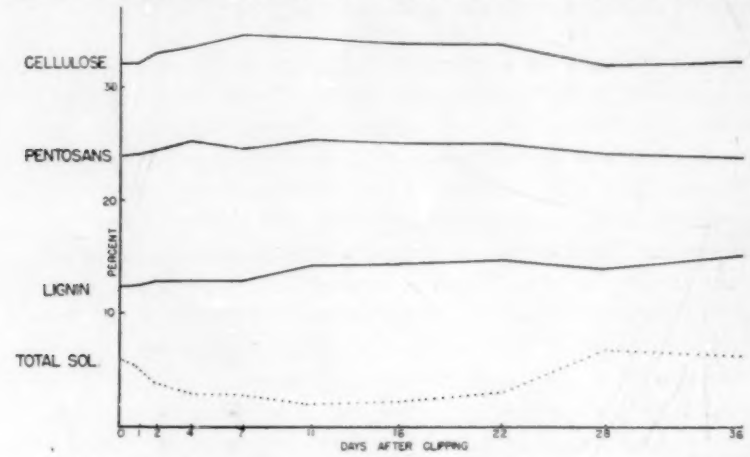

FIG. 5. Cellulose, hydrolyzable pentosans, and lignin (solid lines) and total soluble carbohydrates (dotted line) of roots of ryegrass plants growing in the greenhouse for 36 days after removal of the tops.

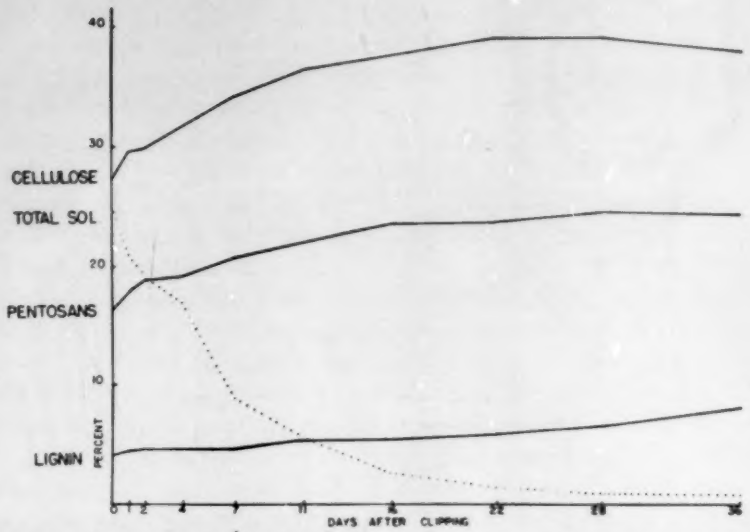

FIG. 6. Cellulose, hydrolyzable pentosans, and lignin (solid lines) and total soluble carbohydrates (dotted line) of stubble of ryegrass plants growing in darkness for 36 days after removal of the tops.

sucrose and 0.41 of glucose; and in the roots, 0.15 per cent. of sucrose and 0.07 of fructose. There was no evidence of utilization of cellulose, pentosan, and lignin.

The changes taking place in several minor carbohydrate constituents are not reported in detail. The oxalic acid hydrolysis liberated reducing sub-

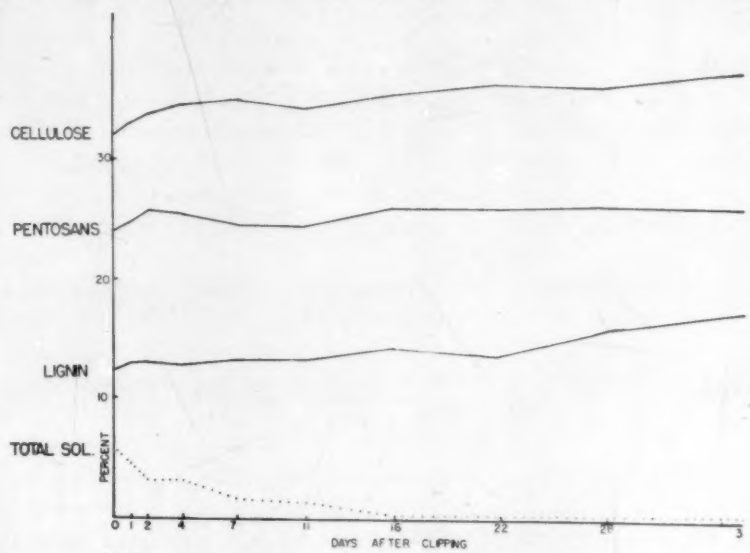

FIG. 7. Cellulose, hydrolyzable pentosans, and lignin (solid lines) and total soluble carbohydrates (dotted line) of roots of ryegrass plants growing in darkness for 36 days after removal of the tops.

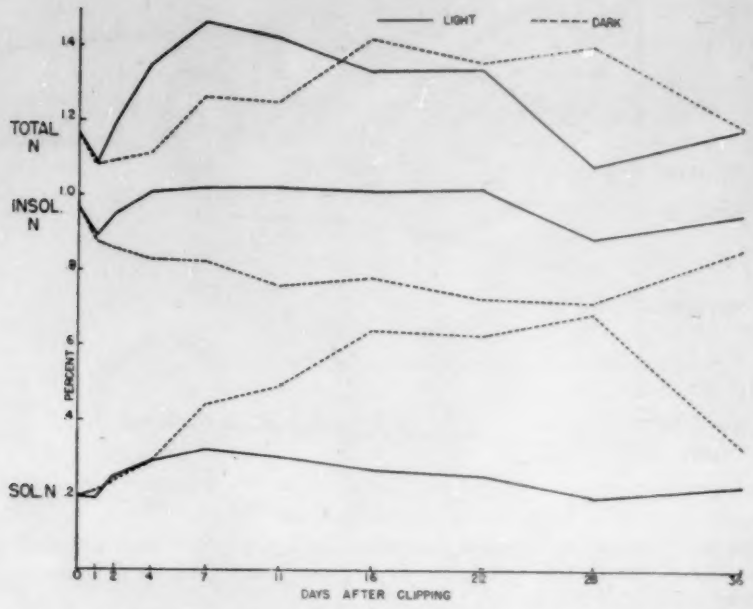

FIG. 8. Total, insoluble, and soluble nitrogen of stubble of ryegrass plants growing in the greenhouse (solid lines) and in darkness (dotted lines) for 36 days after removal of the tops.

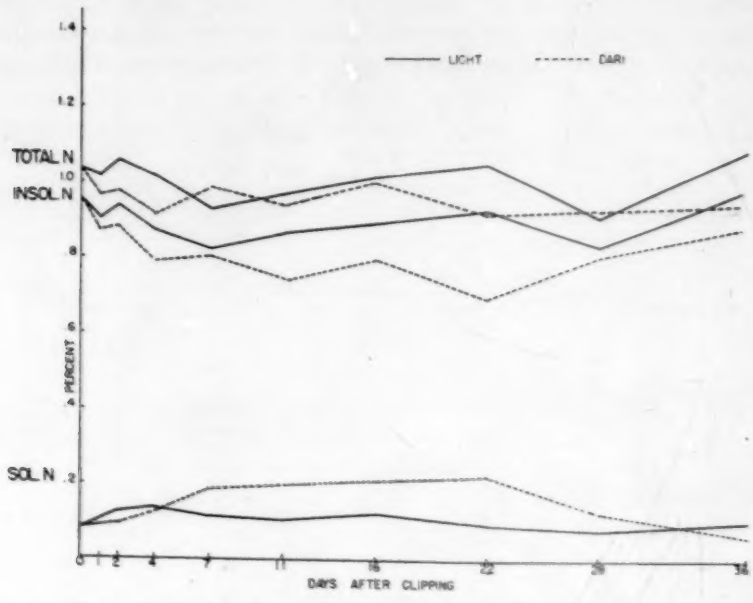

FIG. 9. Total, insoluble, and soluble nitrogen of roots of ryegrass plants growing in the greenhouse (solid lines) and in darkness (dotted lines) for 36 days after removal of the tops.

stances in addition to fructose which have been calculated as glucose. These substances varied between 0.6 and 1.0 per cent. in the stubble and between 0.4 and 0.6 per cent. in the roots. The sulphuric acid hydrolysis also liberated some fermentable, non-fructose substances which have been calculated as glucose and probably include the portion liberated by oxalic acid. The amounts of these components varied between 2.5 and 4.5 per cent. in the stubble and between 1.0 and 2.0 per cent. in the roots. The uncertainty of the method of calculating sugar mixtures makes it unsafe to attach any importance to these figures.

Soluble, insoluble, and total nitrogen changes in the stubble are illustrated in figure 8. Total nitrogen reached its greatest accumulation in plants in light 7 days after cutting and in plants in the dark somewhat later. The proportion of total nitrogen in soluble form increased from 17 per cent. at cutting time to 22 per cent. at 7 days for plants in the light. In plants in the dark the proportion of soluble nitrogen increased rapidly, amounting to 49 per cent. of the total at 28 days. One fourth of the soluble nitrogen at this date consisted of nitrates which were not detected originally but accumulated rapidly in plants in the dark and later decreased at the time the plants were dying.

Nitrogen changes in the roots are illustrated in figure 9. Total nitrogen showed less fluctuation than did that of the stubble. The amount of nitrogen in the soluble form increased from 8.2 to 13.5 per cent. at four days. In the dark the soluble nitrogen reached 21 per cent. of the total at 11 days; 36 per cent. of this was nitrate.

Discussion

The term "reserve substances" in plants has been freely used. "Reserve carbohydrates" usually are referred to as those carbohydrates which vary in amount and are alternately accumulated and utilized by the plant in its growth cycle or when disturbed by changes in its environment. In some cases carbohydrates occurring in the leafy parts of plants are referred to as reserves; but in pasture plants carbohydrates present in the tops, which are removed by grazing, cannot function as reserves for the plant under grazing conditions.

In this paper no attempt will be made to define "reserves." An attempt is made to describe only changes that take place in the plant parts which remain after partial defoliation. The disappearance or decrease in amount of certain constituents of the roots and stubble during the period when new growth is progressing rapidly, indicates that such substances may be properly called reserves.

The results reported here do not include all carbohydrate changes. For example, the acid-hydrolyzable material, often referred to as hemicellulose, has not been broken down into all of its component parts. The major part of it, excluding fructosan, is believed to be pentosan but it may consist of more than one pentose compound. Some non-pentosan material, minor in

quantity, also occurs. As the results indicate, the total acid-hydrolyzable material does not show any considerable utilization. It is possible that some part of it may have functioned as utilizable material for the synthesis of new compounds but if this were so it was replaced in the synthesis of other materials of similar analytical properties.

Complete exhaustion of any particular compound did not occur with the plants growing in light. Since in this experiment not all of the photosynthetic tissue was removed and since new leaf tissue was being produced throughout the course of the experiment under favorable environmental conditions, synthesis of carbohydrates occurred in sufficient quantity to prevent complete exhaustion of the reserves. The degree of clipping to which the plants were subjected was not particularly severe and the carbohydrate content of the plants was high at the time of cutting. If a second cutting had been made at some period a week or two after the first at a time when soluble carbohydrates were at a much lower level, subsequent recovery of the plants probably would have been greatly retarded. Further work is contemplated along this line.

In plants growing in the dark following clipping, quantitative changes in carbohydrates occurred in a similar manner as in plants growing in the light. This supports the observation of GRABER *et al.* (**8**) that the growth of plants in darkness might be used to give an indication of the quantity of reserve substances present. When plants were kept for a long period in the dark so that reserves might be considered completely exhausted the constituents removed were the same as those utilized in considerable quantity by plants in light.

Nitrogen reserves have received only minor attention. A nitrate-containing nutrient solution was furnished the plants at all times and the nitrogen content of the plant did not decrease appreciably. There was, however, an increase in the proportion of soluble nitrogen to total nitrogen. This may suggest that proteins are hydrolyzed to more soluble forms. There is little doubt that in plants growing in the dark there was a marked hydrolysis of proteins in the parts studied.

Summary

Changes in the carbohydrate content of roots and stubble of perennial ryegrass grown in gravel cultures in the greenhouse were determined at intervals during a recovery period of 36 days following partial defoliation.

Water-soluble carbohydrates including glucose, fructose, sucrose, and fructosan decreased rapidly for several weeks and then increased. Cellulose, hydrolyzable pentosan, and lignin failed to show any decrease. The soluble carbohydrates alone gave evidence of being reserve substances.

Plants placed in darkness after cutting underwent changes in composition similar to those in light except that soluble carbohydrates continued to decrease almost to the point of exhaustion.

There is evidence that hydrolysis of proteins took place in plants placed in darkness.

Acknowledgements are due to Dr. T. G. Phillips of the University of New Hampshire for suggestions regarding methods of analysis, particularly the lignin and non-fermentable procedures, and to Miss Martha Jayne for assistance in the analyses.

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EFFECT OF OXYGEN CONCENTRATION ON THE RESPIRATION OF SOME VEGETABLES[1]

HANS PLATENIUS

(WITH SEVEN FIGURES)

Introduction

Commercial methods of storing fruits and vegetables in modified atmosphere are based on the fact that respiration, ripening, and other physiological processes can be retarded by maintaining an atmosphere in which the oxygen content is lower and the carbon dioxide concentration higher than in normal air. Many investigators assume that it is the presence of carbon dioxide rather than the limited oxygen supply which has a depressing effect on the physiological activity of the plant tissue. In fact, modified atmosphere storage is frequently spoken of as "carbon dioxide storage."

There is reason to believe, however, that the importance of a limited oxygen supply itself has been underestimated. Indirect evidence for this view is found in the results of THORNTON (**8**) which make it clear that the presence of carbon dioxide in the storage atmosphere does not always depress the respiration of plant material. On the contrary, he found that the respiration rate of potatoes and onions was markedly increased when held in an atmosphere of normal oxygen content to which varying quantities of carbon dioxide had been added. On the other hand, the same treatment had a depressing effect on the respiratory activity of asparagus and strawberries.

In the literature few experiments are reported which deal exclusively with the effect of low concentrations of oxygen on the respiration of plant tissue. Some of these experiments were conducted for a few hours only, and there is no assurance that the results would have been the same had the storage period been extended to several days. Other studies were carried out in the complete absence of oxygen. Obviously, the course of respiration under anaerobic conditions is entirely different from that which is followed when a low, but constant supply of oxygen is maintained.

Most textbooks merely state that the oxygen concentration of the air can be changed over a wide range without materially affecting the rate of respiration. Evidence cited in a review of the literature by MACK (**5**) indicates that such a broad statement is subject to several exceptions. PARIJA (**6**) observed that the production of carbon dioxide of apples was lowest when the oxygen concentration of the air was about 5 per cent.; it rose to higher levels above and below this critical concentration. STEWARD, BERRY, and BROYER (**8**), working with thin disks of carrot and artichoke tissue suspended in weak salt solutions, observed that aeration of the solution with a gas mixture containing only 2.7 per cent. oxygen markedly depressed the carbon dioxide production of the tissues. On the other hand, raising the

[1] Paper no. 254. Department of Vegetable Crops, Cornell University, Ithaca, N. Y.

oxygen concentration of the aeration stream to 40 per cent. did not result in respiration rates higher than were observed in normal air. More recently CHOUDHURY (**3**) investigated the effect of different oxygen concentrations on the respiration of several vegetables. He found that the respiration rate of potatoes remained fairly constant at oxygen concentrations ranging from 6.2 to 98.6 per cent. Artichokes decreased in respiratory intensity at oxygen levels below normal, and the respiration rate of carrots increased or decreased with corresponding changes in the supply of oxygen.

What little experimental evidence is available points to the conclusion that the effect of low oxygen concentrations on respiration varies with different kinds of plant tissue. This is to be expected considering the wide variations in the normal rate of respiration and the differences in relative surface and permeability to gases of plant material. Moreover, the results are likely to be influenced by the length of exposure, the temperature, and other experimental conditions.

The following experiments were conducted for the purpose of obtaining data which would serve as a basis for developing practical methods of modified atmosphere storage. It is reasonable to expect that any combination of gases in the storage atmosphere which effectively reduces the normal respiration rate also retards the process of deterioration. Carbon dioxide gas in the respiration chamber was kept at a fraction of one per cent. in order to study respiration at low levels of oxygen without the complicating effect of another variable. Oxygen consumption as well as carbon dioxide production were measured because both gases play an equally important rôle in the process of respiration. In particular, it was desired to know how far the oxygen concentration could be reduced without affecting the normal course of respiration.

Methods

All experiments were carried out in constant temperature rooms where fluctuations in temperature did not exceed 2° C. Except for brief periods when readings were taken, the vegetables were kept in the dark. Oxygen consumption and carbon dioxide production were measured simultaneously by a method described in detail in an earlier paper (**7**). Carbon dioxide was absorbed by a normal solution of sodium hydroxide in the bottom of the chamber while oxygen entered the chamber under atmospheric pressure at the same rate as it was utilized by the respiring tissue. At the beginning of each experiment, the chamber was flushed with nitrogen long enough to reduce its oxygen content approximately to the level desired. The exact oxygen concentration was determined by gas analysis at the beginning and end of each experiment. In all instances, the original gas composition was maintained within 0.3 per cent. throughout the experiment.

Readings of oxygen consumption and carbon dioxide production were taken at intervals of 24 hours. All data were expressed on the basis of the original fresh weight. Because of the high humidity in the chambers, there

were practically no losses from transpiration and total weight losses over a period of several days were less than 5 per cent.

Methods approved by the Association of Official Agricultural Chemists (1) were used for chemical analyses. Sugars were determined by weighing the cuprous oxide precipitate. The quantity of soluble nitrogen was found by evaporating the alcoholic extract under vacuum, treating the residue with sodium sulphite, and digesting it with a mixture of sulphuric acid, salicylic acid, and mercuric oxide. Protein nitrogen was determined in the residue from the alcohol extraction, using the Kjeldahl-Gunning-Arnold method.

Vegetables used for these experiments were garden-fresh, harvested less than two hours before each experiment was started. Great care was taken to have comparable samples for storage under different oxygen concentrations. Each lot was divided into smaller ones containing an identical number of individuals and these were as uniform in size and shape as could be obtained by careful selection. According to the size of individual specimens, samples ranging from 100 to 700 grams were used in the respiration studies. In no instance did a sample contain less than 15 specimens.

Results

Asparagus

In the first series of experiments, the gas exchange of asparagus held at a temperature of 20° C. was studied simultaneously at nine different levels of oxygen ranging from 1.0 to 20.5 per cent. In figure 1 the average rates

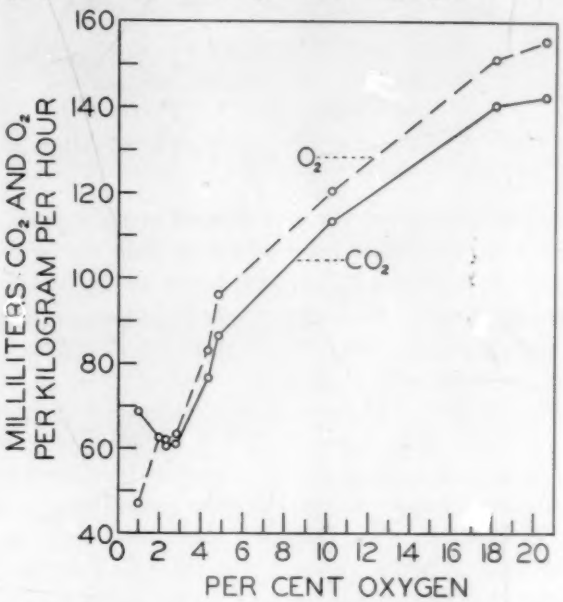

FIG. 1. Average rates of oxygen consumption and carbon dioxide production of asparagus at nine levels of atmospheric oxygen during a three-day period.

of carbon dioxide evolution and oxygen consumption during a three-day interval are plotted against the oxygen concentrations maintained in the different chambers. Attention is called to the fact that the abscissa represents oxygen concentrations, not time intervals. On the ordinate, respiration rates are plotted in terms of milliliters. Using this expression instead of milligrams, deviations from unity in the respiratory quotient can be recognized at a glance by inspecting the relative position of the two curves.

The data show that any change in the oxygen content of the storage atmosphere caused corresponding changes in the respiration rate of the tissue. This effect became increasingly pronounced as lower levels of oxygen were approached. Based on the values for carbon dioxide production,

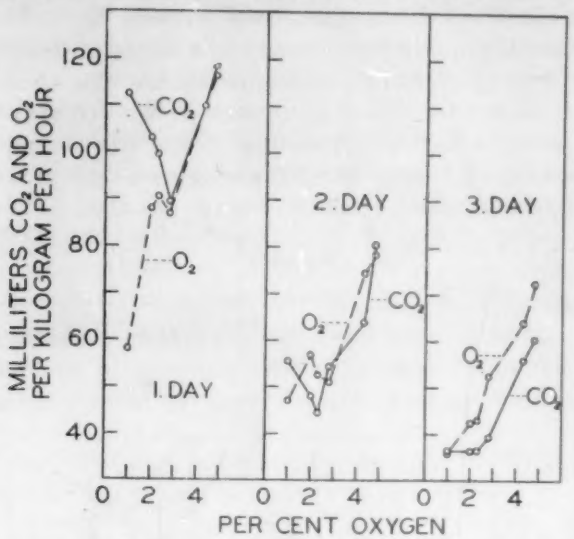

FIG. 2. Average daily rates of oxygen consumption and carbon dioxide production of asparagus at low levels of atmospheric oxygen.

a minimum in respiratory activity was reached at an oxygen concentration of 2.3 per cent. At this point the respiration rate was only 40 per cent. of that occurring in normal air. At still lower levels, carbon dioxide production began to rise again while oxygen consumption continued to fall off sharply. According to the terminology used by BLACKMAN (**2**), 2.3 per cent. of oxygen represents the "extinction point of N R"; it is the concentration at which the type of respiration occurring in pure nitrogen becomes extinct. Above the extinction point of N R sufficient oxygen is available to maintain aerobic respiration and to suppress fermentation completely. Below this point aerobic respiration still takes place but diminishes rapidly in intensity. Fermentation, on the other hand, increases; at a concentration of one per cent. oxygen, the combined output of carbon dioxide from aerobic and anaerobic respiration becomes considerably larger than it is at the extinction point.

In figure 2 the respiration rates for the range from 1.0 to 4.8 per cent. of oxygen are plotted separately for each of the three days. Following the usual drift that occurs with time, the respiratory activity declined at any one oxygen concentration during successive days. It is also evident that the position of the extinction point shifted with time. During the first day, it was located at 2.8 per cent.; during the second, at 2.3 per cent.; and during the third day it had dropped to 1.0 per cent. This shift of the extinction point is probably associated with the normal decline in respiratory intensity as time progresses. At any rate, it shows that the tissue becomes more tolerant to low oxygen concentrations as the storage period is lengthened.

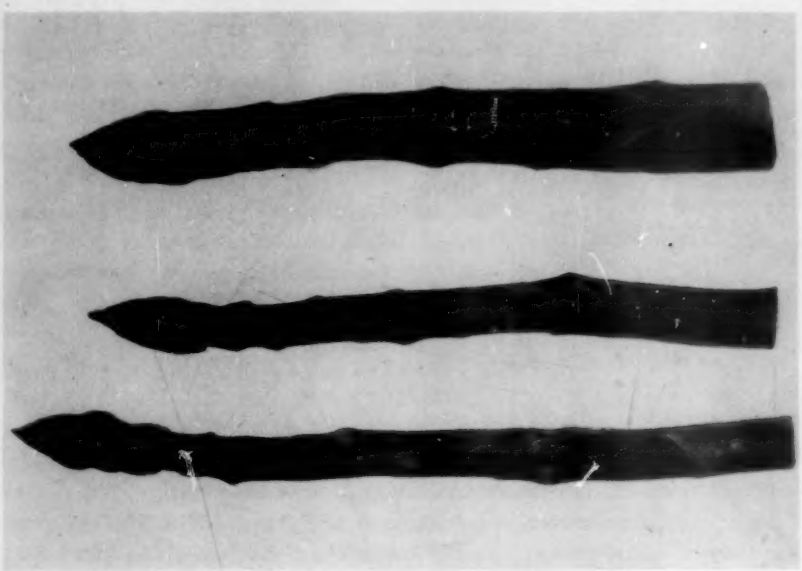

FIG. 3. Injury to asparagus stalks caused by anaerobic respiration at concentrations of atmospheric oxygen below 2.8 per cent.

There was evidence that anaerobic respiration at low levels of oxygen resulted in the accumulation of toxic end products of incomplete oxidation. This was indicated by definite signs of injury of the tissue which appeared after one day of storage in all the samples held at oxygen concentrations of less than 2.8 per cent. As shown in figure 3, sections of the external tissue had collapsed, forming deep longitudinal channels in the upper portion of the spears. These symptoms of injury were identical with those that are produced when asparagus is covered with a fairly thick coat of wax or when spears are exposed to temperatures just above the freezing point for several weeks. Aside from visible injury, the samples held at one and two per cent. oxygen had a pronounced musty and alcoholic odor at the time the experiment was discontinued. It is possible that the injury to the tissue caused

changes in the permeability to gases. This would explain the secondary rise in oxygen consumption in the range between 2.0 and 2.8 per cent. oxygen concentration which was observed during the first and second day of storage. The sharp rise in carbon dioxide production with decreasing levels of oxygen likewise may be attributed to increased permeability of the tissue, although the principal reason for this rise is to be sought in conditions becoming more favorable for rapid anaerobic respiration.

The practical significance of reduced respiration at low levels of oxygen is that losses in carbohydrates, which in asparagus consist chiefly of sugars, are greatly reduced. This conclusion is substantiated by the results of chemical analyses taken at the beginning and end of the storage period. Table I shows that after three days of storage in normal air, asparagus had

TABLE I

EFFECT OF OXYGEN CONCENTRATION IN THE ATMOSPHERE ON TOTAL SUGARS, PROTEIN AND SOLUBLE NITROGEN OF ASPARAGUS HELD AT 20° C. DATA ARE EXPRESSED ON THE BASIS OF ORIGINAL FRESH WEIGHT

OXYGEN CONTENT OF THE STORAGE ATMOSPHERE	STORAGE PERIOD	TOTAL SUGARS	SUGARS RETAINED*	PROTEIN NITROGEN	SOLUBLE NITROGEN
%	*days*	%	%	%	%
.....	0	2.32	100.0	0.315	0.093
20.5	3	1.39	59.9	0.244	0.129
10.2	3	1.56	67.2	0.249	0.146
4.8	3	2.09	90.1	0.260	0.149
2.8	3	2.13	91.8	0.283	0.125
2.0	3	2.01	86.6	0.253	0.154

* Percentage of original.

lost 40 per cent. of its original sugar content whereas only eight per cent. had been lost at an oxygen concentration of 2.8 per cent. In other words, sugar losses from asparagus under low oxygen were only one-fifth of those occurring in normal air.

Comparing the respiration data with the results of the chemical analyses, it became apparent that in any one treatment sugar losses account for only one-half to two-thirds of the total quantity of carbon dioxide evolved. The same observation was made by the writer in earlier experiments (7), and at that time the suggestion was offered that part of the substrate used in respiration of asparagus is furnished by the products of protein hydrolysis. The present data confirm this assumption. There was a definite decrease in protein nitrogen with a corresponding rise in soluble nitrogen during storage. Proteolysis was most active in the sample stored in normal air, it was somewhat slower at low concentrations of oxygen.

Essentially the same trends in respiration rates were observed when asparagus was stored at a temperature of 10° C. at three levels of oxygen. Because of technical difficulties, records were obtained only for carbon dioxide production. The results of this experiment (table II) show that

TABLE II

RESPIRATION RATES OF ASPARAGUS STORED AT 10° C. AND AT THREE LEVELS OF OXYGEN FOR SEVEN DAYS

OXYGEN CONTENT OF THE STORAGE ATMOSPHERE	MILLILITERS CARBON DIOXIDE PER KILOGRAM PER HOUR					
	1ST DAY	2ND DAY	3RD DAY	4TH AND 5TH DAY	6TH AND 7TH DAY	AVERAGE FOR SEVEN-DAY PERIOD
%	*ml.*	*ml.*	*ml.*	*ml.*	*ml.*	*ml.*
20.5	86.3	68.9	61.9	52.8	53.6	61.4
2.1	57.5	40.8	51.9	41.7	28.5	41.5
1.2	44.2	28.3	28.6	20.3	25.2	27.4

the respiration rate could be reduced as much as 55 per cent. by lowering the oxygen content of the storage atmosphere to 1.2 per cent. Significant is the fact that 1.2 per cent. oxygen in the air was sufficient to maintain aerobic respiration at a temperature of 10° C. After seven days, the samples showed no signs of injury nor was there any odor of alcohol perceptible in the chambers. Also, the fact that the carbon dioxide production at 1.2 per cent. oxygen was considerably lower than it was at 2.1 per cent. indicates that at a temperature of 10° C. the extinction point of N R lies close to 1.2 per cent. oxygen or even lower.

SPINACH

The respiration rates of spinach held for three days at a temperature of 20° C. were measured at three levels of oxygen. The results are expressed graphically in figure 4. Obviously, the number of points definitely located is too small to show more than a general trend in the slope of the curves. It is apparent, however, that changes in the oxygen concentration above five per cent. had little effect on carbon dioxide production. Only at an oxygen level of 0.8 per cent. was a significant reduction in respiration

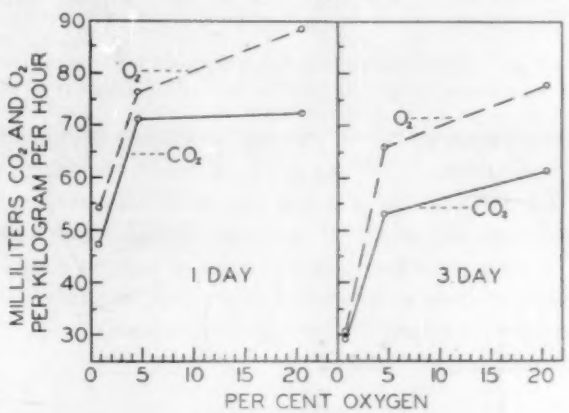

FIG. 4. Rates of oxygen consumption and carbon dioxide production of spinach at three levels of oxygen during the first and third day of storage.

rate obtained. At that concentration, carbon dioxide output had decreased to 65 per cent. on the first, and to 48 per cent. on the third day of storage. In no instance did carbon dioxide production exceed oxygen consumption, although the respiratory quotient showed a tendency to become larger at lower levels of oxygen.

Spinach in normal air has an unusually low respiratory quotient. This may be due to the continuous formation of oxalic acid or other organic compounds of high oxygen content or it may be caused by the decomposition of proteins and their utilization as substrate in respiration. Whatever the reaction involved may be, a lowering of the oxygen content of the storage atmosphere seems to inhibit that process. It is of practical significance that an oxygen concentration of 0.8 per cent. reduced respiration to nearly one-half its normal rate, but was sufficient to maintain normal aerobic respiration. The sample held in that particular gas mixture showed no signs of injury; in fact, it was definitely superior in appearance and taste to that stored in normal air.

Snap beans

As shown in figure 5, the response of snap beans to low concentrations of

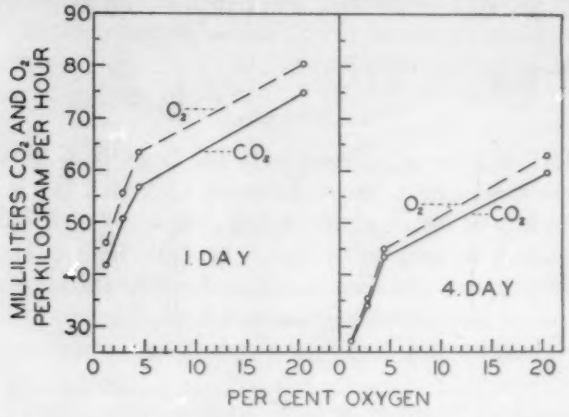

Fig. 5. Rates of oxygen consumption and carbon dioxide production of snap beans at four levels of atmospheric oxygen during the first and fourth day of storage.

oxygen at a temperature of 20° C. was similar to that of spinach. Reducing the oxygen content of the air to 4.4 per cent. had little effect; but at a concentration of 1.2 per cent. the average carbon dioxide evolution during the four-day period was only about 50 per cent. of that which was produced in normal air. Within the experimental range of oxygen concentrations, the respiratory quotient always remained below unity, and this is in accord with the fact that none of the samples developed symptoms of injury which could be traced to anaerobic respiration.

Shelled peas

The respiration curves for shelled peas at a temperature of 20° C. differed in several respects from those of other vegetables. Figure 6 shows

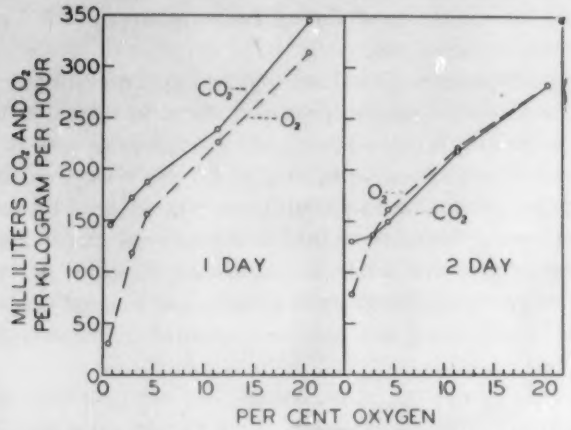

FIG. 6. Rates of oxygen consumption and carbon dioxide production of shelled peas at five levels of oxygen during the first and second day of storage.

that within the experimental range a nearly linear relationship existed between carbon dioxide production and the oxygen content of the air. The respiratory quotient was well above 1.0 at all concentrations during the first day but dropped to a level close to unity a day later. Only at the lowest oxygen level did carbon dioxide production exceed oxygen consumption consistently.

Fermentation occurred in the samples held at oxygen concentrations of 0.8 and 2.8 per cent. This was evident from the strong alcoholic odor of these particular samples and from the pronounced off-flavor before and after cooking. Moreover, the surface of these peas showed a brown discoloration, and the seed coat of some had burst. Although there can be no doubt that in these samples fermentation occurred simultaneously with aerobic respiration, the combined production of carbon dioxide from these two processes did not cause a secondary rise in carbon dioxide output at extremely low oxygen levels as had been observed in asparagus. It is almost impossible, therefore,

TABLE III

EFFECT OF OXYGEN CONCENTRATION IN THE ATMOSPHERE ON TOTAL SUGARS OF SHELLED PEAS HELD AT 20° C. DATA ARE EXPRESSED ON THE BASIS OF ORIGINAL FRESH WEIGHT

OXYGEN CONTENT OF THE STORAGE ATMOSPHERE	STORAGE PERIOD	TOTAL SUGARS	SUGAR RETAINED*
%	*days*	%	%
.....	0	6.43	100.0
20.5	2	1.76	27.4
11.4	2	3.09	48.1
4.4	2	4.46	69.4
2.9	2	4.44	69.1

* Percentage of original.

to establish the exact location of the extinction point of N R from inspection of the respiration curves alone.

In commercial practice beneficial results can be expected from storing peas under low oxygen tension, provided the concentration remains sufficiently high to prevent fermentation. At a temperature of 20° C., the best results were obtained at an oxygen level of 4.4 per cent. At this concentration, the average carbon dioxide production was reduced 56 per cent. The degree to which sugar losses were inhibited was even more pronounced than the respiration data would indicate. As shown in table III, the control lot had retained only 27 per cent. of its original sugar content while 69 per cent., that is, more than 2½ times as much, was retained in the sample kept at 4.4 per cent. oxygen.

Carrots

The respiratory behavior of carrot roots, illustrated in figure 7, changed considerably during the six-day storage period. The maximum reduction

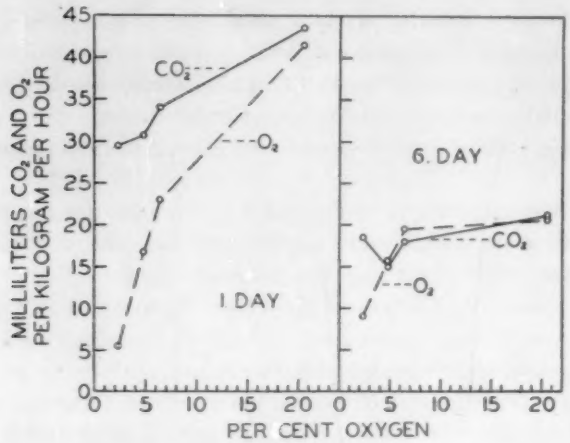

Fig. 7. Rates of oxygen consumption and carbon dioxide production of carrots at four levels of oxygen during the first and sixth day of storage.

in carbon dioxide production observed within the experimental range remained close to 30 per cent. regardless of the fact that the respiratory intensity of all samples declined steadily as time progressed. In the beginning, the respiratory quotient ranged from 1.05 in normal air to 5.8 at 2.3 per cent. oxygen. Gradually the respiratory quotient became smaller, and on the sixth day, two of the samples had reached values slightly below unity. Only at the lowest oxygen concentration did the quotient remain above 2.0 at all times. After the first day, a secondary rise in carbon dioxide production was observed at oxygen levels below 4.8 per cent. This rise persisted; in fact, it became more pronounced during the latter part of the storage period.

Only the sample held at an oxygen concentration of 2.3 per cent. appeared to be permanently injured by toxic end-products of anaerobic respi-

ration. This particular sample had lost its turgor and showed all the other symptoms of injury from an insufficient oxygen supply. The carrots kept at an oxygen level of 4.8 per cent., the next higher concentration employed, remained in sound condition in spite of the fact that the initial respiratory quotient was as high as 1.8.

In this connection, it should be mentioned that in another experiment carrots were kept in excellent condition for six months when stored at a temperature of 2° C. in an atmosphere containing one to two per cent. oxygen. This serves as additional proof that the danger of injury from anaerobic respiration is lessened as the storage temperature is lowered.

Discussion

In analyzing the results of respiration experiments, one must keep in mind that the measured rate of gas exchange deals only with two compounds of the entire chain of reactions that make up the complex process of respiration. Without quantitative chemical analyses showing which organic constituents serve as substrates and which compounds are formed as intermediate and end products, it is impossible to gain a clear understanding of the mechanism of plant respiration. Even if data of such a detailed study were available for one kind of plant tissue, it would be a fallacy to assume that different species or different organs of the same plant have the same respiratory mechanism. Moreover, there is sufficient evidence to show that the course of respiration of any one tissue may change materially with variations in temperature and other environmental conditions. Interpretations of the experimental data of this study are made with these limitations in mind.

An examination of the data on oxygen absorption shows that within the experimental range any decrease in the concentration of atmospheric oxygen produced a corresponding drop in the rate at which oxygen was consumed. This relationship was by no means a linear one. An increment in the oxygen content of the air caused a greater increase in oxygen consumption at low levels of oxygen than it did at levels close to normal. Also, the response to changes in oxygen concentration was more pronounced in the beginning of the storage period than later. Finally, the magnitude of the response varied with different types of tissue.

These observations lead to the conclusion that in the five vegetables examined, oxygen becomes a limiting factor of respiration whenever its concentration drops below that of normal air; and the extent to which the utilization of oxygen is reduced depends on the partial pressure of the gas in the air, the relative respiratory intensity in normal air, and possibly to the permeability of the tissue to oxygen.

The data for carbon dioxide production can be interpreted correctly only if the corresponding values of oxygen are taken into account. The rôle of oxygen in respiration is two-fold: It is a reactant in normal aerobic respiration, and it inhibits anaerobic anabolism. The second function of oxygen is

known as the PASTEUR effect. As defined by DIXON (4) it is the action of oxygen on living cells which reduces carbohydrate destruction and suppresses or diminishes the accumulation of the products of anaerobic metabolism. The PASTEUR effect comes into play whenever the storage atmosphere contains small quantities of oxygen but complete depression of anaerobic respiration is not attained until a certain threshold concentration is reached. BLACKMAN (2) has called this threshold concentration the extinction point of N R, that is the concentration of oxygen at which the course of respiration followed in an atmosphere of pure nitrogen ceases to exist. Below the extinction point, carbon dioxide production, as measured experimentally, consists of the combined gaseous end products of aerobic and anaerobic respiration. Depending on the intensity of anaerobic respiration, the values for total carbon dioxide output may actually increase below the extinction point. This was found to be true in asparagus during the first and second day and in carrots after the first day of storage. A similar secondary rise below the extinction point was observed by BLACKMAN (2) in apples held at low concentrations of oxygen. In these tissues the extinction point of N R is obviously close to the oxygen concentration at which total carbon dioxide production is at a minimum.

It is more difficult to determine the position of the extinction point in plant tissues which fail to show a secondary rise in carbon dioxide production. A sudden increase in the respiratory quotient may indicate the beginning of anaerobic respiration, but it must be remembered that a quotient above unity in itself cannot be taken as evidence that fermentation is taking place. The respiratory curves of shelled peas, for instance, show a respiratory quotient considerably higher than unity at all concentrations within the experimental range; but injury from anaerobic respiration was apparent only in the samples held at oxygen levels of less than three per cent. It is evident, therefore, that the exact location of the extinction point cannot always be determined by inspection of the respiration data. In fact, for practical purposes the appearance of symptoms of injury and the presence of an alcoholic odor in the chamber seem to be a safer and more sensitive index of how far the oxygen content of the storage atmosphere can be lowered without harmful effects.

Important is the fact that different plant tissues vary considerably in their tolerance to low levels of oxygen in the atmosphere. Oxygen concentrations of less than one per cent. were sufficient to maintain normal aerobic respiration in spinach and snap beans while shelled peas did not tolerate levels below four per cent. Also, there was evidence that the tolerance to low oxygen increases with the aging of the tissue or with a lowering of the storage temperature.

The degree to which the rate of respiration could be reduced varied with different vegetables. In some, the reduction exceeded fifty per cent.; in others it was barely forty per cent. for the entire storage period. Certainly, these results conflict with the statement commonly made that the oxygen

content of the air has little influence on the respiratory rate of plant tissue. In fact, the magnitude of the effect of low oxygen storage on respiration suggests that the benefit from modified atmosphere storage, in which part of the oxygen is replaced by carbon dioxide, is due chiefly to the limited oxygen supply rather than to the presence of carbon dioxide. Additional experimental data are needed to determine quantitatively the effect that these two gases have on the respiration rate of various fruits and vegetables.

On the basis of results obtained thus far, it appears that holding fruits and vegetables in an atmosphere of low oxygen content offers possibilities as a practical method of storage. It has been shown that in some vegetables the retention of sugars is even greater than could be expected from the respiration data. Moreover, recent unpublished data have demonstrated that low oxygen is equally effective in retarding the destruction of ascorbic acid.

Several limitations and technical difficulties must be recognized. Aside from the fact that low oxygen storage requires gas-tight storage rooms, provisions have to be made for the continuous removal of carbon dioxide. In order to avoid injury from anaerobic respiration and to obtain maximum benefits, the oxygen concentration in the atmosphere must be maintained within a narrow range, the exact range being specific for each crop and each temperature. It also should be pointed out that many fruits and vegetables spoil because of storage diseases long before an appreciable loss in carbohydrates has occurred. In the course of recent experiments, it was frequently observed that molds and bacteria grow equally well at an oxygen concentration of one per cent. as in normal air. Even the presence of 20 per cent. carbon dioxide in the atmosphere was ineffective in controlling the spread of storage diseases. Consequently, beneficial results from low oxygen storage can be expected only for those crops which are highly perishable because rapid respiration or oxidation of ascorbic acid are important factors in deterioration.

Summary

Five vegetables, asparagus, spinach, snap beans, shelled peas, and carrots were held in respiration chambers in which part of the oxygen was replaced by nitrogen. A definite concentration of oxygen within the range of 0.8 to 20.5 per cent. was maintained in each chamber. Average daily rates of oxygen consumption and carbon dioxide production were determined.

The critical oxygen concentration below which the tissue was injured by anaerobic respiration (extinction point of N R) was about one per cent. for spinach and snap beans, 2.5 per cent. for asparagus, and four per cent. for peas and carrots when held for several days at 20° C. There was evidence that the tissue became more tolerant to low oxygen with aging and with a lowering of the storage temperature.

By choosing the most effective oxygen concentration, the respiration rate as measured by carbon dioxide production could be reduced about 50 per

cent. At the most effective oxygen level, the respiration rate of asparagus was 40, and that of carrots 65, per cent. of the rate in normal air.

Sugar losses occurring in the course of respiration were determined for asparagus and peas. At the most effective levels of oxygen, asparagus retained eight times and peas two and one-half times as much sugar as comparable samples in normal air. It was shown that in asparagus proteins furnish about one-third of the substrate of respiration.

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GERMINATION, GROWTH, AND RESPIRATION OF RICE AND BARLEY SEEDLINGS AT LOW OXYGEN PRESSURES

J. VLAMIS AND A. R. DAVIS

(WITH FIVE FIGURES)

An extreme sensitivity to oxygen deficiency is demonstrated by barley seeds undergoing germination. The presence of a film of water above the seeds, for example, inhibits their germination and rapidly leads to fermentation of a pronounced character with a rapid disintegration of the tissues. This mortality may be partly reduced by forcing a stream of air through the water.

On the other hand, it is a common agricultural practice to germinate rice seeds in standing water 1 to several centimeters in depth. This behavior of rice has been observed on several occasions but with one recent exception, no attempt has been made to establish the oxygen relations of germinating rice on a quantitative basis.

In an exhaustive review of the literature on oxygen relations of plants (**6**), the general effects on the respiration of a wide variety of plants show an independence of oxygen until a partial pressure of 2 per cent. oxygen is reached. Below this point a very sharp decline follows in respiration, measured in most experiments by carbon dioxide production.

MACK (**6**) germinated wheat seeds aerobically and after 42 hours transferred the seedlings to 12 different oxygen tensions ranging from 0.6 per cent. to 98.3 per cent. and covering five temperatures from 10° C. to 30° C. at 5 degree intervals. The carbon dioxide evolved was measured at several intervals within a 46-hour period and the shoot lengths determined at the end of this time. The anticipated inter-dependence of temperature and oxygen was found. The respiration curves for each of the 3 highest temperatures, oxygen taken as the variable, assume parallel shapes differing only as to absolute values. The effect on shoot growth is greater than that on respiration in response to temperature change, and to oxygen at the lowest pressures. The maximum in respiration occurs uniformly at 30° C., and the shoot growth attains a maximum between 20° and 25° C.

TAKAHASHI (**11**) germinated rice in oxygen-free water but growth was limited to a small amount of plumule development which ceased after reaching a length of 3 cm. YOKOI (**14**) likewise obtained only shoot growth of rice under water, and in sand cultures the relative growth of the radicle increased at the expense of the shoot as the water content of the sand declined. These observations have been repeated by NAGAI (**10**). The germination of rice on the surface of submerged soil has been found to be superior to that of seeds placed below the soil (**5**).

The catalase activity in barley, wheat, and rice has been compared in aerobic and anaerobic atmospheres (**7**). The catalase activity was shown to

decrease in aerobically germinated seeds. Bermuda grass and cat-tail have been found to have a superior germination under reduced oxygen pressure (**8, 9**).

Respiratory quotients of barley and several types of rice seeds germinated in air and water have been found to vary considerably (**1**), but the existing oxygen pressures in the environment were not determined.

A recent comparison of wheat and rice showed a rapid decline in growth and in oxygen consumption for both types of seeds as the partial pressure of oxygen was lowered (**12**). The total carbon dioxide production likewise declined for wheat but increased with rice and reached a maximum as oxygen pressure approached zero. These results were obtained on seedlings germinated aerobically for brief periods and placed under the experimental conditions for intervals ranging from 1 to 96 hours.

Results

Oxygen consumption of seedlings germinated aerobically

The seeds used throughout were Sacramento barley, and Carreon Upland rice from the Philippines. Seeds were germinated aerobically between 2 screens, of which the lower was covered by cheese cloth, and the upper by mosquito netting. Moisture was maintained by wick action of the cloth dipping into a pan of water. The details of the procedure have been described previously (**4**).

Germination occurred in a dark room controlled at 25° C. When the seedlings attained a 2-cm. shoot length, they were placed in experimental vessels of the Fenn apparatus (**2**) to measure the oxygen consumption under six oxygen pressures.

Mixtures of oxygen and nitrogen were prepared such as to give partial pressures of 9.5, 5.2, 2.7, 1.0 and 0.2 per cent. oxygen. Atmospheric air provided 21 per cent. oxygen.

The analysis for oxygen was performed by the Winkler method (**13**), and a colorimetric procedure based on oxidation of metallic copper to blue cupric ion (**3**).

For each measurement the seedlings were introduced into the vessel and the oxygen uptake measured at 21 per cent. oxygen until equilibrium was established, usually within one hour. Then one of the gas mixtures was introduced and the oxygen uptake measured for that pressure of oxygen. This provided a method of relating all rates of respiration to those found at 21 per cent. which arbitrarily received a value of 100. All determinations were made in triplicate at 23.5° C.

The curves for rice and barley seedling respiration (oxygen consumption) are shown in figure 1 and appear very similar, giving a steep trend from 0.2 per cent. to 9.5 per cent. oxygen.

Carbon dioxide production of seedlings germinated aerobically

The respiration of seedlings similar to those above was also compared with respect to carbon dioxide production at 21 per cent. and 0.2 per cent.

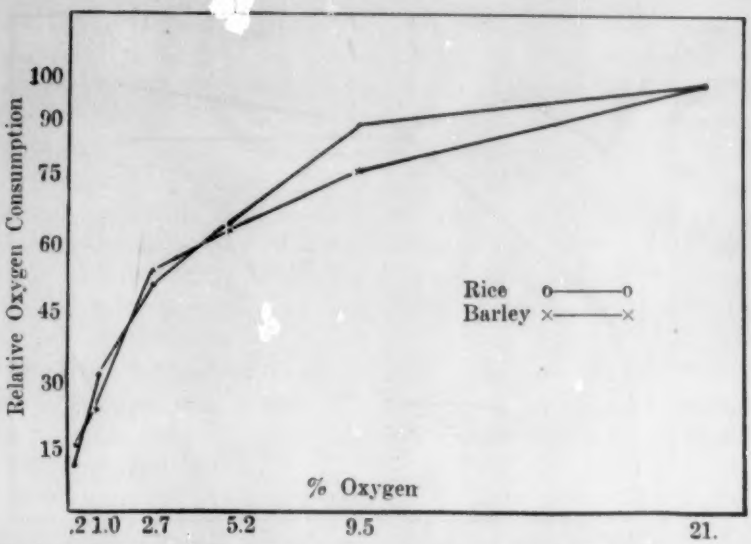

FIG. 1. Relative oxygen consumption under different oxygen tensions of rice and barley seedlings germinated aerobically.

oxygen. This was done by passing the CO_2-free gas under pressure at the rate of 1 liter per hour through a half-liter Erlenmeyer flask containing 25 barley or rice seedlings. A double layer of filter paper at the bottom of the flask retained sufficient moisture to maintain a saturated atmosphere. The time required for a series of determinations was less than 12 hours. The temperature was maintained at 25° C.

The carbon dioxide released was absorbed in KOH towers and titrated to the endpoint of phenolphthalein. The data (table I) show the reduction in respiration of rice and barley seedlings under 0.2 per cent. oxygen. For both plants this amounts to about 50 per cent. of the CO_2 evolved under 21 per cent. oxygen.

GERMINATION AND GROWTH AT DIFFERENT OXYGEN TENSIONS

The preceding experiments measured the oxygen sensitivity of barley and rice seedlings germinated under the usual aerobic atmosphere. A

TABLE I

ANAEROBIC CO_2 PRODUCTION AT 25° C.

TYPE OF RESPIRATION	BARLEY	RICE
Aerobic (21% oxygen)	100	100
Anaerobic (0.2 % oxygen)	50.0	49.5
	54.5	49.0
	52.6	56.5
	51.5	50.0
	52.5	53.8
Average anaerobic	52.2	51.8

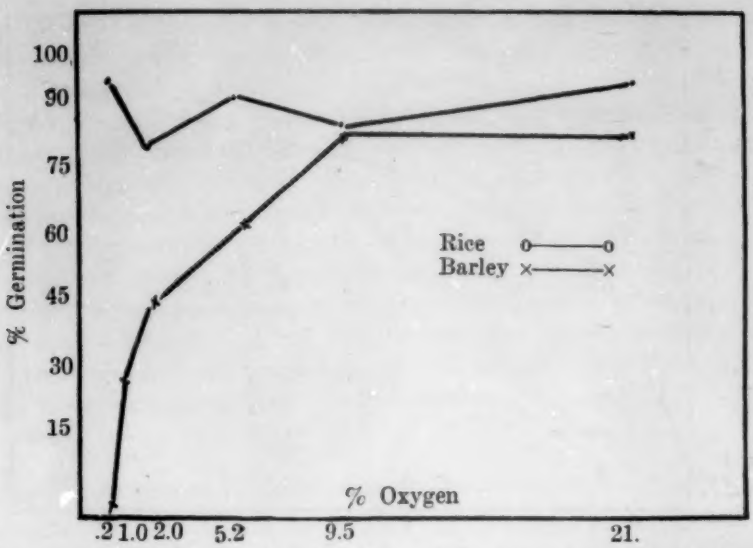

FIG. 2. Germination of rice and barley seeds in various mixtures of oxygen and nitrogen renewed every 24 hours.

further comparison was undertaken to germinate seeds under several partial pressures of oxygen from the earliest stage of germination.

Twenty-five dry seeds were placed on 2 filter papers in each of six 500-ml. Erlenmeyer flasks equipped with inlet and outlet tubes. Each flask was filled with distilled water saturated at the required oxygen pressures.

After 18 hours the water was displaced by a known oxygen-nitrogen mixture under pressure, leaving sufficient water to maintain the filter moist,

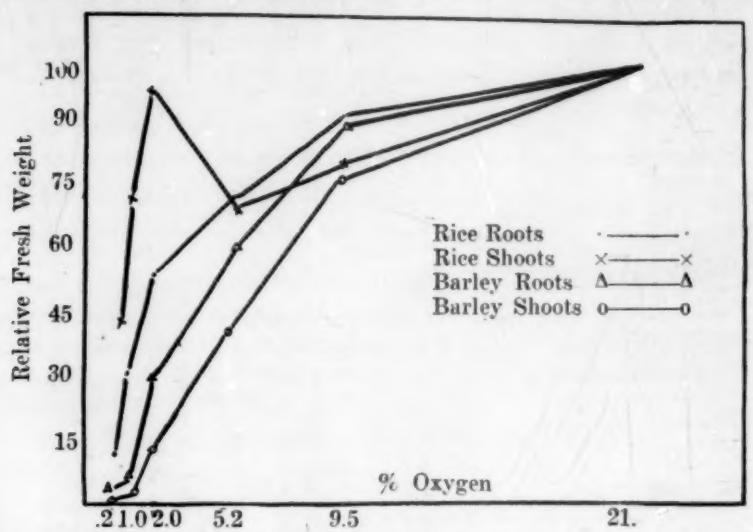

FIG. 3. Relative root and shoot fresh weight in oxygen-nitrogen mixtures renewed every 24 hours.

while avoiding any seed submersion. The atmosphere thus provided was renewed every 24 hours so that a fresh supply of the gas mixture was available once daily. The temperature was kept constant at 25° C.

Barley seedlings were harvested after a period of 5 days, while the rice was grown for 11 days. This difference in time was imposed by the slower rate of development in rice.

The percentage of germination for barley (fig. 2) is not unlike the oxygen consumption curve of barley seedlings germinated aerobically (fig. 1). The germination of rice shows a contrasting reaction to low oxygen pressure, and the percentage of seed germinated is independent of oxygen tension.

The growth values (fig. 3) are based relatively on those at 21 per cent. oxygen which are taken as 100. The root growth curves of rice and barley are very similar although rice does show a slightly higher trend than barley at the lower oxygen values.

The shoot growth of barley is of the same character as the root response, whereas rice shoots show as much growth in 2.0 per cent. oxygen as in 21 per cent.

An unsatisfactory feature of this experiment is to be found in the static atmosphere. Even with daily renewals the accumulation of some carbon dioxide and the inevitable reduction of the initial oxygen content represent a certain uncontrolled variation.

Germination, growth, and respiration

In order to meet the objections described above, seeds were germinated in continual streams of gas mixtures with known oxygen content. This automatically provided a means of measuring respiration by absorbing in alkali tubes the carbon dioxide evolved.

The culture chamber again consisted of a half-liter Erlenmeyer flask containing two filter papers and a rubber stopper with 2 glass tubes. On the inlet side provision was made for elimination of any CO_2 in the incoming gas by absorption in strong alkali. The outlet tube led to the standard alkali absorption tower. The rate of aeration equalled 1 liter per hour, which was adequate to maintain the oxygen levels for all mixtures except the 0.2 per cent. (anaerobic) level.

Seeds were placed in the Erlenmeyers and soaked overnight in water aerated with the respective oxygen mixtures. After forcing the liquid from the flasks, the respiration measurements were started immediately and observations made at frequent intervals continuously until the termination of the experiment. Barley was harvested after 5 days and rice after 11 days.

The relative fresh weights are illustrated in figure 4, and show in an accentuated form the data obtained in the preceding experiment. Barley root and shoot growth rapidly declines as the oxygen pressure goes below 9.5 per cent. The rice roots have a slightly lower rate of decrease than barley shoots or roots, but the difference is not enough to be of great significance.

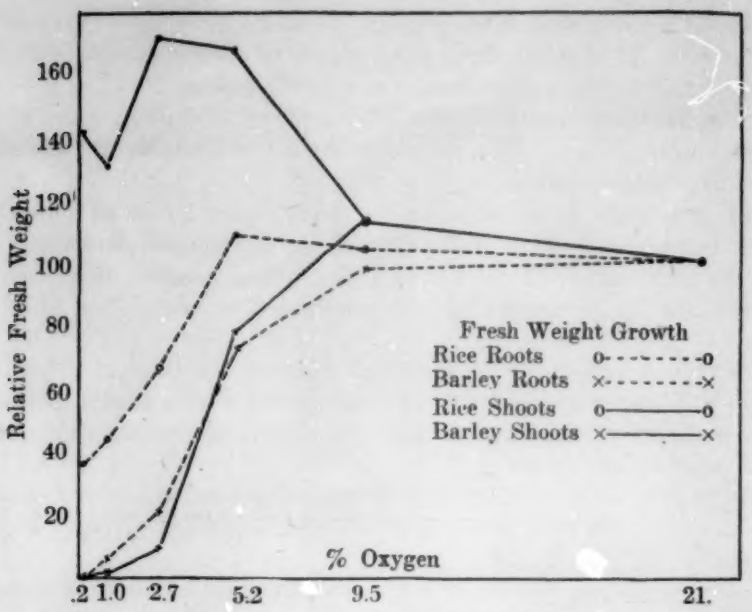

FIG. 4. Relative root and shoot fresh weight in continuous streams of oxygen-nitrogen mixtures.

The rice shoots at all pressures ranging from 0.2 per cent. to 5.2 per cent. oxygen are considerably larger than at the 2 highest pressures. In

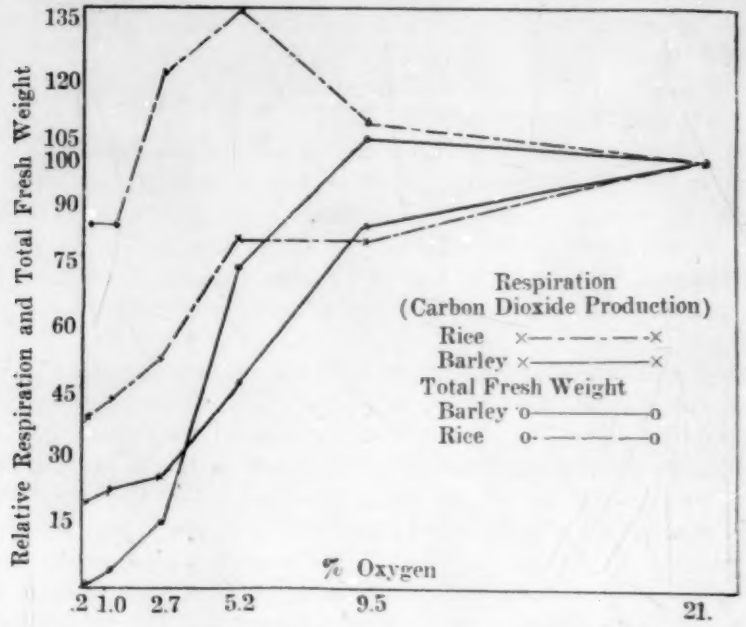

FIG. 5. Total carbon dioxide evolved and total fresh weight in continuous streams of oxygen-nitrogen mixtures.

fact, the highest oxygen tension (21 per cent.) actually yielded the minimum value in shoot growth, although the difference from the next value (9.5 per cent. oxygen) is not very large.

The respiratory quantities express the total carbon dioxide production throughout the germination and growth of the seedlings (fig. 5). The barley curve follows the previous patterns while that of rice is slightly higher than barley at the four lowest oxygen pressures. Some such relation would be anticipated on the basis of the differences found in growth at low oxygen tensions, especially with reference to the rice shoots.

The total growth is also presented to show the relation of total respiration to the combined root and shoot growth. Further calculations to estimate the respiration per unit growth were not made in the absence of any assessment of the respective respiratory power of the shoot and root under low oxygen tensions.

It is obvious that the rice seedlings germinated under low partial pressures of oxygen exhibit a dual character. This expresses itself on the one hand by the usual inhibiting effect on the development of the roots which is comparable to that occurring in barley root and shoot growth. On the other hand is found an apparent stimulation of the shoot system which results in a considerable increase over that undergoing aerobic germination.

This dichotomy can hardly be explained by any such simple device, for example, that has implied an increased availability of some reserve substances which are released to the shoots through the inability of the roots to utilize them. In a similar category is the prospect that a substance is produced by the roots under oxygen deficiency and increases the growth of the shoot at low oxygen pressures. Neither explanation provides a clue as to the origin of the unique behavior of the shoot system in the first place.

Any attempt to elucidate the mechanism as its primary aim must measure the anaerobic response of root and shoot separately without sacrificing too greatly in regard to time, the inter-relationships involved. In addition to the oxygen and carbon dioxide quantities exchanged, the effects of some anaerobic intermediates and end-products likewise require exploration in the hope of identifying the metabolic chain responsible for the behavior of the rice shoot at low oxygen tension. Possible toxicity effects on the shoots at the higher pressures also merit some attention.

Summary

Barley and rice seeds germinated aerobically were tested for respiratory behavior over short time intervals under partial pressures of oxygen ranging from 0.2 per cent. to 21 per cent. For both plants there is an equally rapid decline in oxygen consumption as the pressure goes below 9.5 per cent. Measured by carbon dioxide production, the respiratory activity of barley and rice at 0.2 per cent. oxygen is approximately half that at 21 per cent.

Seeds were germinated under various oxygen pressures and continuously aerated with oxygen-nitrogen mixtures up to the time of harvesting. The carbon dioxide released throughout this time was measured periodically.

Barley root and shoot growth is considerably diminished below 9.5 per cent. oxygen. Rice roots undergo a smaller reduction in growth rate than barley roots. Rice shoot growth is at a minimum at 21 per cent. and increases with declining oxygen pressure, reaching a maximum at approximately 3 per cent. Below this pressure the growth of rice shoots is still significantly higher than at 21 per cent.

The rate of evolution of carbon dioxide by rice decreases with lowered oxygen pressure and that by barley declines more rapidly.

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FIRMNESS OF STRAWBERRIES AS MEASURED BY A PENETROMETER

LELAND BURKHART

(WITH TWO FIGURES)

Introduction

Strawberries are subjected to compression, bruising, and shearing effects during harvesting, packing, and shipping. The resistance of the fruit to mechanical injury is related to firmness; hence there is needed a suitable method for measuring firmness in order to evaluate varietal differences, stage of maturity, or degree of ripeness, effects of environmental conditions during fruit development on firmness, and effects of different shipping and storage conditions on changes in firmness.

The improved types of pressure testers (**6, 10**) designed for pomaceous fruits are not sufficiently sensitive for evaluating differences in firmness of strawberries. No satisfactory pressure tester has been reported for use on strawberry fruit (**6**). Resistance of strawberries to wounding was measured by HAWKINS and SANDO (**8**) as the pressure required to puncture the epidermis with a needle 636 microns in diameter. CULPEPPER *et al.* (**3**) measured the resistance of the strawberry to penetration by a small needle (0.032 inch in diameter). The depth of penetration was not indicated, however; the sudden penetration of the needle was used as the end point. This needle tester gave an index of maturity but did not measure varietal differences in firmness or resistance to crushing in shipping or handling. This device did not give a measure of the resistance of the first to deformation or flattening as does the pressure or squeeze tester developed by HALLER *et al.* (**7**). This tester which measured the resistance to flattening of the fruit is a modification of the type described by VERNER (**14**). A 2,000-gram scale was used and the plunger disc moved through a distance of $\frac{1}{8}$ inch. Full ripe berries of approximately the same stage of maturity were used. A direct correlation was found to exist between the dry weight and firmness of the different varieties.

In order to determine the effect of moisture on the softness of berries, KIMBROUGH (**9**) used a sweet-corn tester, with a plunger $\frac{1}{4}$-inch in diameter which registered in grams the pressure needed to penetrate the berry. Berries from watered plants were larger and softer than from the unwatered plants.

SHOEMAKER and GREVE (**13**) adapted an apple pressure tester to strawberries. A plunger $\frac{1}{4}$-inch in diameter was used. Readings were taken when the plunger had been forced $\frac{3}{8}$-inch into the berry at a point one third the distance from the calyx to the apical end. The end point of penetration was indicated by electrical contacts. Firmness increased through the season, June 11th to 16th, and this was attributed to the smaller size of the berry.

CLARK (4) used a pressure tester with a plunger $\frac{5}{16}$-inch in diameter for ripe berries. Force was applied by hand until the plunger penetrated $\frac{1}{8}$-inch into the berry. DARROW (5) used this tester and found that fertilizers had no effect on firmness of strawberries.

One of the chief disadvantages of these previously reported pressure testers is that the applied force is not mechanically controlled.

Strawberry penetrometer

The improved type of penetrometer as shown in figure 1 and described herein is designed to include a controlled application of the pressure necessary for fruit penetration by the plunger. The pressure was applied by the delivery of 30-mesh zinc metal from a funnel burette, at a uniform rate of 10 grams per second, to a container resting on the platform of a spring

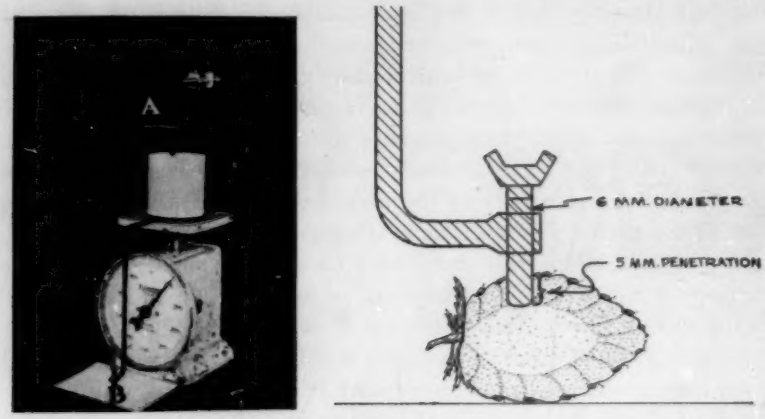

FIG. 1. Left: Photograph of the penetrometer showing controlled uniform rate of application of force at A which is transmitted to the strawberry fruit placed under the plunger at B. The force is applied as 30-mesh zinc metal delivered from the funnel burette at A. Right: Diagram showing fruit penetration by the penetrometer plunger.

balance of 1000-grams capacity. The applied force is transmitted from the platform to the fruit plunger 6 mm. in diameter by means of a steel rod fastened to the platform. On the basis of the structure of the fruit and observations that tissue breakdown occurs initially in the cortical region of the fruit, a penetration of 5 mm. proved satisfactory in the vulnerable zone of greatest diameter which is one-third the distance from the calyx to the apical end. The distance of 5 mm. traversed by the plunger into the fruit is conveniently indicated by the deflection of the pointer on the dial from the zero reading of 100 to the dial reading 250. The dial reading of 250 is the endpoint of the determination obtained by immediately stopping the flow of zinc by releasing the pinchcock. The firmness of the fruit is indicated by the weight of zinc required to overcome the resistance of the fruit to plunger penetration which is obtained after subtracting the resistance of the balance to the passage of the plunger through air.

Results

Variations in the chemical characteristic ascorbic acid (**2**) in sun-ripened and shade-ripened strawberries suggested the possibility that wide variation might occur in firmness as a physical characteristic of fruit grown under these different light conditions. There is shown in table I the effect of direct illumination and shading of foliage on large, medium, and small Klondike strawberries. The fruit was picked for uniformity as to visual red color. Strawberries ripening in direct sunshine tend to be more firm than the shade-ripened fruit, and this tendency is consistent in the large, medium, and small fruits. Under these conditions of sunshine and shade, the larger

TABLE I

STRAWBERRY PRESSURE TESTS. VARIATION IN FIRMNESS WITHIN AND BETWEEN SUN-RIPENED AND SHADE-RIPENED KLONDIKE STRAWBERRIES PICKED FROM AN EXPERIMENTAL FERTILITY PLOT*

	Berry 1	Berry 2	Berry 3	Berry 4	Berry 5	Berry 6	Mean
	F†	F	F	F	F	F	F
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
Sun-ripened							
Large	90– 96	128–143	135–167	143–159	148–155	158–181	142.9
Medium	80–128	100–128	134–164	140–150	148–158	148–160	137.0
Small	70– 72	68–100	148–159	148–170	150–154	166–178	131.8
Shade-ripened							
Large	64– 91	86–110	97–107	117–125	131–160	161–174	118.5
Medium	76– 86	72– 98	86– 96	94–102	114–124	180–188	109.6
Small	68– 70	78– 80	88– 94	96–110	102–118	110–130	95.3

* Picked May 9; analyzed May 10.

† Firmness of each berry is indicated by two determinations made at a right angle to each other in the same transverse plane.

berries appear more firm than the smaller ones, which is not in agreement with the findings of DARROW (**5**). The largest variations in firmness occurred, however, between individual fruits under both light conditions and within a fruit as shown in table I. A more detailed report of the factors associated with these wide variations between fruits and within a fruit will appear in a later publication. These variations in firmness within typical samples of different varieties are shown in table II and the need of further study of the internal variations in samples is suggested. The mean values of firmness indicate wide varietal differences. It is possible that firmness values greater than 200 grams may indicate better shipping and keeping qualities than values below 200 grams. The possibilities of the penetrometer for measuring quantitative differences in firmness of new seedling strawberries developed in breeding programs is indicated. Seedling 3911–13 has a mean firmness value 200 per cent. greater than seedling 3805–06.

Firmness of strawberries as related to stages of maturity or degree of ripeness on the basis of visual red color intensification is shown in figure 2. In the early stages of red coloration, firmness of the fruit decreased sharply.

TABLE II

VARIATION IN FIRMNESS WITHIN AND BETWEEN RIPE STRAWBERRIES OF MEDIUM SIZE IN TYPICAL SAMPLES OF DIFFERENT VARIETIES*

VARIETY	BERRY 1	BERRY 2	BERRY 3	BERRY 4	BERRY 5	BERRY 6	MEAN
	F†	F	F	F	F	F	F
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
Seedling 3803–06	58– 70	68–114	84– 88	88–108	68–134	132–144	96.5
Klondike	100–106	102–108	104–118	113–121	124–136	130–132	116.1
Missionary	110–133	122–129	128–150	152–159	158–165	178–180	147.0
Blakemore	232–248	232–252	212–270	232–276	228–302	252–278	251.1
Seedling 3911–13	234–276	250–264	266–274	298–318	302–338	344–350	292.8

* Picked April 29, 1942; analyzed April 30, 1942.

† Firmness of each berry is indicated as two determinations made at a right angle to each other in the same transverse plane.

After reaching the pink stage the Klondike variety decreased more rapidly than the Fairmore. The firmness of the Fairmore variety is more stable during ripening than the Klondike variety. A marked varietal difference in firmness of plums as related to maturity on the basis of color change has been reported (**7**). In connection with the evaluation of stages of maturity on degrees of ripeness, there is also needed a quantitative measurement of the red color or anthocyanin concentration. As the strawberries reach an over-ripe condition, the internal tissue of the fruit breaks down, resulting in rupture of the skin and it is at that stage when the ascorbic acid content of the fruit is rapidly destroyed (**10**).

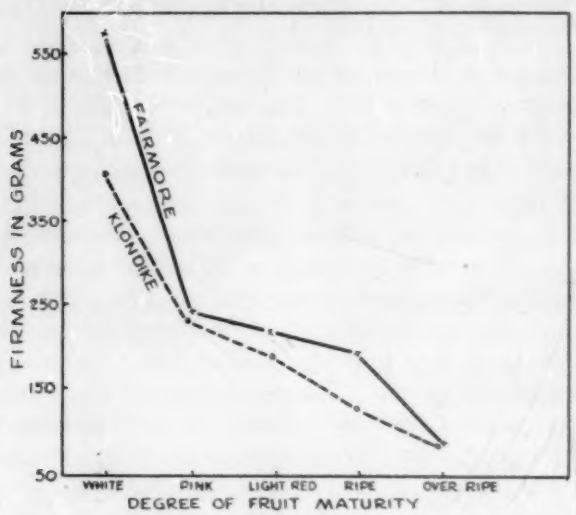

FIG. 2. Firmness of Fairmore and Klondike varieties of strawberry fruit at different stages of fruit maturity as indicated by red color intensity.

The use of the penetrometer in evaluating effects of storage conditions on keeping and shipping quality of strawberries is suggested by the firmness values shown in table III. The low storage temperature increased firmness, and in this connection HAWKINS and SANDO (8) found that low temperatures increased the resistance of the strawberry epidermis to puncturing. The need of a refined sampling technique to increase the reliability of the firmness determinations for evaluating treatment differentials is indicated by the variation in the mean values of the respective samples which received the same fertilizer treatment.

TABLE III

EFFECT OF STORAGE TEMPERATURE ON FIRMNESS OF RIPE BLAKEMORE STRAWBERRIES SAMPLED FROM AN EXPERIMENTAL FIELD*

	APRIL 25	APRIL 27 AFTER 48 HR. AT 8° C.	APRIL 27 AFTER 48 HR. AT 27° C.
	gm.	*gm.*	*gm.*
Sample A	188.5	234.6	132.1
Sample B	195.6	227.4	115.8
Sample C	204.5	230.5	125.8
Sample D	196.5	217.1	128.1
Mean	*196.0*	*227.5*	*123.0*

* Picked April 24. Each value represents the mean of twelve determinations.

Summary

1. The improved penetrometer for measuring the firmness of strawberries is designed to include a controlled uniform rate of application of the force necessary for fruit penetration by the plunger. A penetration depth of 5 mm. with a plunger 6 mm. in diameter proved satisfactory. The firmness of the fruit is indicated by the force required to overcome the resistance of the fruit to penetration by the plunger.

2. Strawberries ripened in sunshine tend to be more firm than shade-ripened fruit. Larger berries appeared more firm than the smaller.

3. Varietal differences in firmness are indicated and ranked in the following descending order: Blakemore, Massey, Missionary, and Klondike. Two new seedling varieties differed in firmness to the extent of 200 per cent.

4. Changes in firmness of Klondike and Fairmore strawberries during ripening are indicated.

5. Wide variations in firmness occurred among fruits within samples and within individual fruits. These observations indicate the need of a refined sampling technique to increase the reliability of the firmness determinations for evaluating treatment differentials. The use of the penetrometer in evaluating changes in firmness under different storage conditions is indicated.

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SPECTROGRAPHIC ANALYSIS OF PLANT ASH FOR SEVERAL ELEMENTS SIMULTANEOUSLY[1]

R. C. Nelson, P. C. Hamm, and Y. S. Tsiang

(WITH TWO FIGURES)

Introduction

For studies of the relative mineral nutrient absorptive capacities of different plant species or strains under varying environmental conditions, a method of determining all the elements of interest in a single series of operations is a considerable convenience. The nature of quantitative spectroscopy suits it well to the solution of such problems. Important difficulties arise, however, in the standardization of the variations in burning the sample and making the photographic record; and in the uncertainties due to the interaction of the elements of the sample. The first of these problems is ordinarily met by the use of an internal standard, and the second by comparison of the unknown with preparations of similar composition made artificially. In the matter at hand, both of these devices are unduly laborious.

Theoretical considerations

Internal standardization usually involves the addition of a known quantity of an element not present in the sample, and the use of lines so obtained as density standards for the estimation of unknown quantities of other elements. This succeeds only when the line of the standard and the line of the unknown which is compared with it are activated under the same conditions and are affected similarly by variations in the arc and in composition of sample; and when the two elements in question volatilize simultaneously during the burning of the sample. The first condition may be met by a pair of lines which are produced by corresponding shifts in their respective atoms. The second may be tested by the use of moving plate spectrograms, which reveal the relative times at which the elements in the sample pass into the arc.

These conditions may usually be met, but often only with difficulty, and the use of a separate standard for each of six elements in an analysis would be troublesome. In a spectrograph of medium dispersion such a procedure would lead to a crowded spectrum, owing to the lines of the extraneous elements introduced.

At a sacrifice of some accuracy, and with the additional loss either of economy or sensitivity, this difficulty may be overcome by dispensing with the internal standard, and altering the other conditions of the analysis so as to reduce the variability or compensate for it. Brunstetter, Myers, Wilkins, and Hein (**1**) accomplished this by adjusting the conditions of

[1] Paper no. 2072, Scientific Journal Series, Minnesota Agricultural Experiment Station.

the burning of the sample so that they were reproducible, with a concomitant loss of sensitivity.

The instrument used in the present experiments, that described by NELSON (3) is not sufficiently sensitive to permit this solution, but its economy of operation as regards photographic materials is such that it is possible to increase the number of exposures made of each sample without making the cost of a determination unduly large. By making four, or even eight exposures for the highest accuracy, it is possible to average out the variations caused by irregularities in the arc, without requiring a great expenditure of either time or money.

The interactions of elements in the arc and the effects of concentration of one upon the density of lines of another are not yet well understood. In

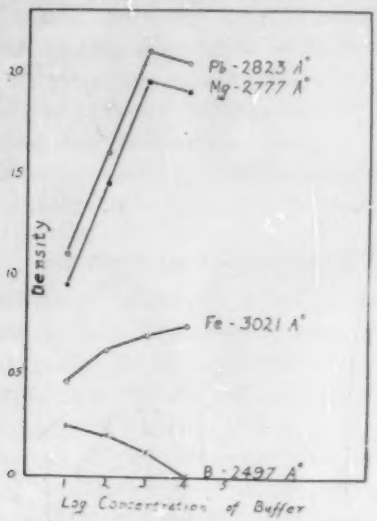

FIG. 1. Effect of sodium acetate on line density.

the analysis of plant ash the chief difficulty arises from differences in the quantity of sodium and potassium salts present. Figure 1 shows the effect of the addition of increasing amounts of sodium acetate upon the density of certain lines. Increasing the amount of sodium in the sample, under the conditions of these experiments, enhances the density of certain lead and magnesium lines, has a small effect upon a line of iron, and depresses a boron line. Other elements may also cause such effects or be similarly affected. It is thus apparent that the composition of the artificial standard from which the calibration curve is obtained must closely approximate that of the unknown sample. To make such a standard by trial and error would be very laborious. A more convenient method, using the unknown itself to obtain the calibration curve will be described.

Let x be the concentration of an element in the unknown, expressed in micrograms per milliliter. By taking a series of different volumes of the unknown solution, containing V_1x, V_2x, etc., micrograms of the element, it

is possible to obtain a calibration curve in terms of x. If, now, a known quantity of the element is added to V_1x, the density of the line will be increased to an extent corresponding to some determinable point on the calibration curve, V_nx, or $V_1x + y$ micrograms $= V_nx$, giving an equation which may be solved for x, after which numerical values may be attached to the proportional points of the calibration curve. By this means, the procedure is standardized, using a preparation which is almost identical in composition with the unknown. When a large number of samples of similar composition is being analyzed, a composite of several of them may be used as the unknown in this method. Known amounts of all the elements which are present only in small quantities may be added at once, although obviously

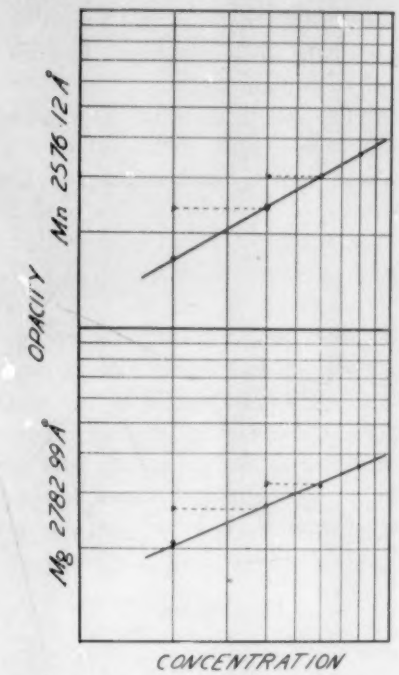

FIG. 2. Standardization curves for magnesium and manganese.

it is preferable not to add potassium to the unknown when standardizing it for the other elements. The value of V_n may be obtained graphically or by solution of an equation fitted to the line density-volume values.

Figure 2 illustrates this method. A curve was made by plotting line density against logarithm of volume of solution taken, using 0.02, 0.04, 0.06, and 0.08 ml. of the unknown and the following equation fitted by the method of least squares.

$$2 + \log V = 0.005 + 1.61 \times \text{density}$$

Then, to 0.02 and to 0.04 ml. of the unknown were added 0.4 micrograms of manganese each. This addition resulted in line densities of 0.366 and 0.480, respectively, corresponding to volumes of 0.0392 ml. and 0.0598 ml. These

data correspond to concentrations of manganese in the sample of 20.8 and 20.2 micrograms per ml., which is good agreement. It is thus possible to perform a spectrographic analysis of an unknown for several elements in a series of simple operations without the necessity of preparing an artificial standard.

Experimentation

The determinations herein described were made on a replica grating spectrograph of medium dispersion, using a DC arc of 40 volts drop across the arc and 25 amperes. The record was made upon Verichrome roll film which was developed 105 seconds in 1:1 D–72 at 18° C., stopped in dilute acetic acid, and fixed with F–5. Although it would have been possible to use a sector disk at the stigmatic focus, it was found that gamma could be controlled well enough by care in development, and no attempt was made to measure the gamma of each film.

One-gram samples of dry plant material were ashed, and the ash extracted overnight at room temperature with 5 ml. of 3 N hydrochloric acid. 0.04 ml. of this solution was used for each exposure. Electrodes were purified according to the method of ZEITLOW, HAMM, and NELSON (**4**), after a cup 2 mm. deep and 4.5 mm. diameter had been drilled at one end of each electrode. This cup was first treated with a drop of a five per cent. solution of paraffin in toluene, after which the sample to be analyzed was pipetted into it and dried under a heat lamp. Electrodes were held three-sixteenths of an inch apart during burning, and arcing was continued for four seconds after the sample was all vaporized, as judged by the sound of the arc. Line densities were read using a densitometer designed for use with roll film and constructed in this laboratory.[2] Four exposures were made of each sample. Occasional exposures which were obviously weak across the whole range of wavelengths, indicating a gross derangement of the arc during burning, were eliminated by inspection, and not considered in calculation of results.

Discussion

The methods here described were developed specifically for application to the analysis of 36 clonal lines of common bromegrass (*Bromus inermis* Leyss.) which were being studied by Y. S. TSIANG. Results of this work will be published later, but some purely analytical aspects of this study will be mentioned here. The following table shows the mean squares of errors due to the analytical procedure compared with the mean squares of total experimental error, computed according to the method of analysis of variance (**2**).

ELEMENT	WAVELENGTH OF LINE	TOTAL ERROR	SPECTROGRAPHIC ERROR
Fe	3020 Å.	220.79	2.46
Cu	3247	13.52	8.23
Ca	3158	1,654,441.0	111.0
Mn	2576	155.67	0.88
K	3446	1,151,410.0	215.0
Mg	2782	39,111.0	257.0

[2] NELSON, R. C., and HAMM, P. C. Unpublished.

It will be seen that the error in determination of copper is relatively large, owing probably to the difficulty of removing copper from the carbons in the purification process. BRUNSTETTER *et al.* also found this to be true. Boron and aluminum might also have been determined, but were not of interest; knowledge of the phosphorus content would have been valuable, but phosphorus was not present in large enough quantity to permit reliable determination. There were seventy-two samples of each of which four exposures were made, using nine rolls of V–620 film having thirty-two exposures per roll.

These procedures have been used in other similar problems and have proved quite satisfactory. It must always be remembered, however, that each type of spectrograph and each type of sample require especially adapted procedures to secure the best results.

Summary

A method is described for determining a number of elements simultaneously in ash samples using an increased number of exposures, simple density measurements without internal standardization, and a procedure for establishing a calibration curve without the use of an artificial standard. This technique was adapted to the analysis of samples of brome grass for iron, manganese, copper, calcium, potassium, and magnesium.

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DECOMPOSITION OF THE LEAVES OF SOME FOREST TREES UNDER FIELD CONDITIONS[1]

FELIX G. GUSTAFSON

(WITH ONE FIGURE)

In the main the few studies that have been made on the decomposition of leaves have either been made under highly artificial conditions (**5**) or else over a short period of time (**1, 3, 4**). Therefore an experiment intended to supply natural conditions as nearly as possible and lasting a number of years was set up in the fall of 1934.

Leaves of sugar maple, hickory (several species), white oak and black oak (several species) were collected from the ground during the period of leaf fall; needles from the red pine were collected from the tree, using needles ready to fall. Weighed quantities of air dry leaves were placed in wire baskets $24 \times 24 \times 6$ inches made from galvanized wire. Half-inch mesh was used for all leaves except pines for which quarter-inch mesh was used to keep the needles from falling out. All baskets were supplied with a cover made from the same material as the rest of the basket. The baskets were placed in the field on November 17. The pine needles were placed under the trees from which they had been collected. The others were placed in a mixed hardwood stand. Before the baskets with the leaves were put in place all fresh leaves were removed from the ground so that they rested on partly decayed leaves. The baskets were placed on level ground to insure that all parts came in intimate contact with the soil. Besides the single species or mixture of species as indicated above, the oak leaves and pine needles were mixed in the order of 72 per cent. of the former to 28 per cent. of the latter. This was done to simulate mixed stands of hardwood and conifers, and to discover whether there is any foundation for the general belief among foresters that there is more rapid decomposition when pine and hardwood leaves occur together than when alone.

The amount of leaves placed in each basket was not the same except in pine, because the leaves were put in weighed baskets first and then weighed. The pine needles were weighed separately and 400 grams put into each basket. The amount of the other leaves varied from about 200 to 300 grams per basket. This quantity of leaves was undoubtedly larger than that which would normally have accumulated on an area of four square feet during one season. The oven dry weight of corresponding samples of leaves were made so that every year comparisons could be made between oven dry leaves.

At the time of setting up the experiments it was thought that maple and hickory leaves would decay very rapidly and therefore only four baskets were made up of these leaves, whereas eight baskets of the others were prepared. The original intention was to remove a basket each spring and fall,

[1] Paper from the Department of Botany of the University of Michigan, no. 822.

but it was soon discovered that if this were done the supply would be used up long before the leaves had completely decayed; therefore, longer intervals were used in later years.

Due to the difficulty of having the baskets made, about a month elapsed between gathering the leaves and placing them out, and during this period they remained in an air dried condition. As there was no appreciable rain until November, there was perhaps little or no delay in initiation of decay during the first fall.

In the fall of 1935 large meshed wire netting was placed over the leaves in each basket and fresh leaves of the proper type put on top to simulate the fall of new leaves. No more leaves were added after this.

Considerable difficulty was encountered in excluding new pine needles as they could easily fall through the top of the basket. The presence of the top layer of needles helped to some extent but undoubtedly needles were included at times which had not been part of the original material. Some difficulty was also encountered in later years with other species as after the top layer of leaves had undergone considerable disintegration it was not possible to remove every bit of these leaves as some would fall through the coarse netting. Another difficulty was the introduction of soil to the bottom layer by worms working up through the soil, and also the growth of grass and other plants through the leaves. All possible care was exercised but undoubtedly the later collections contained material not included at the beginning, and it is believed that none of the original material was lost except through decay so that the loss recorded is somewhat less than it really was. In some instances tunnels of rodents were found under the baskets which would tend to reduce the decay because of lack of contact between the leaves and the soil. This was especially noticeable in the spring of 1942. The samples brought into the laboratory in the fall of 1937 were lost in the process of drying, by getting too hot and catching fire.

The results are presented in table I. The pine and two hardwoods are also presented graphically in figure 1.

TABLE I

DECOMPOSITION OF LEAVES IN PERCENTAGE OF THE ORIGINAL MATERIAL

PLANT	MAY 18, 1935	OCT. 12, 1935	MAY 24, 1936	OCT. 23, 1936	JUNE 2, 1937	OCT. 28, 1938	APRIL 26, 1942
	%	%	%	%	%	%	%
Hickory	15.0	37.4	53.1*	37.0	.....	.....	.....
Sugar maple	17.2	30.0	26.9	31.9	.....	.....	.....
White oak	9.0	16.6	37.4*	28.4	20.0	24.5	63.3
Black oak	10.5	19.9	23.5	24.9	27.4	24.8	46.1
Oak and pine	13.3†	24.8	23.1	26.4	28.0	44.9	59.6
Red pine	5.7	19.7	18.4	21.9	24.3	38.3	.....

* These figures are way out of line but there is no adequate explanation for them.

† In this sample the oak and pine leaves were weighed separately and loss calculated separately. The pine lost 8.25 per cent. and the oak 16.3 per cent., which are greater losses than when they were in separate baskets.

It will be noticed that during the first year there was a greater loss than during any succeeding year. This was no doubt due to the decay of the parenchyma tissue. As had been expected, the hickory and maple leaves decayed much more rapidly than those of the oaks; pines, on the other hand, decayed very slowly. No information on the composition of hickory and maple leaves has been found, but WAKSMAN states that oak and pine have a high hemicellulose, cellulose, and lignin content, which would make them very resistant to bacterial action.

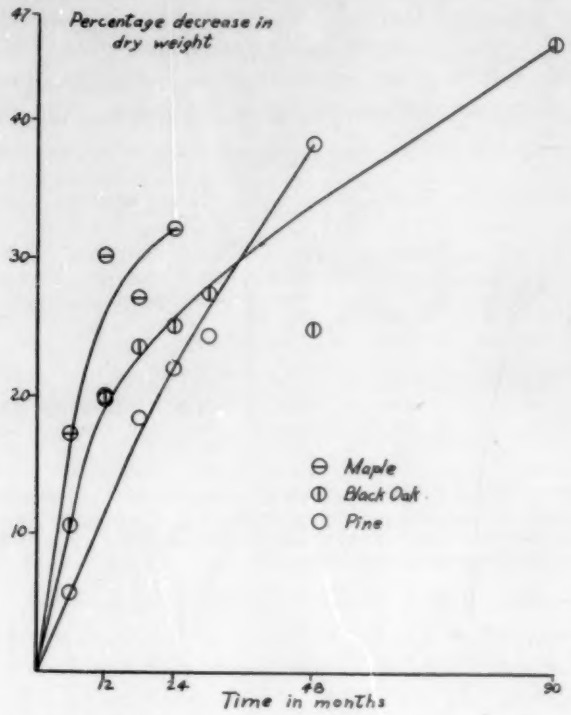

FIG. 1. A graph showing the progressive decomposition of the leaves of red pine, sugar maple, and black oak.

In the 1942 collection the black oak remnant consisted mostly of petioles. The white oak material was composed of recognizable leaf material as well as some petioles. The mixture of pine and oak had the pine needles well disintegrated, and so were the oak leaves.

Figure 1 brings out a difference in the loss of material between the hardwood leaves and the pine needles which is surprising, but easily explained. In the former, the slope of the curve flattens out after the first few collections, whereas the curve for the pine continues at about the same slope. The early rapid disintegration in the hardwood leaves is due to the less resistant parenchyma tissue; but this leaves behind the veins and petioles composed extensively of lignified cells, which decay much more slowly. The

pine on the other hand does not have an extensive parenchyma system distinct from the veins; and the rate of decay is never very rapid, but continues at much the same rate.

The data favor the assumption made by foresters that a mixture of coniferous and hardwood leaves hastens the decay of both. It was not possible, except in the first lot, to determine separately the loss in the two components; but this showed that both the pine and oak leaves lost more weight than when they were in separate baskets. The percentage loss is also greater, year by year, than the oak and pine separately. As an explanation for this might be offered the fact that decaying pine needles produce an acid reaction, which would suppress bacterial activity, but increase fungal activity. The presence of oak leaves with their high calcium content (**2**) would neutralize the decomposing material, and both bacteria and fungi would be active. The two acting together might decompose more material than fungi or bacteria alone.

Summary

While the experiment is admittedly imperfect, it nevertheless gives us some new information concerning the decay of some hardwood leaves and pine needles over a period of several years. It shows that such leaves as sugar maple and hickory decay much more rapidly than oak, and that oak leaves decay rapidly as long as parenchyma is concerned, but the large veins and petioles last many years. A mixture of pine and hardwood leaves increases the rate of decay of both kinds of leaves.

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INTERNAL PRECIPITATION OF PHOSPHORUS IN RELATION TO ALUMINUM TOXICITY[1]

KENNETH E. WRIGHT

Various investigators (**2, 4, 5, 6, 7, 8, 10, 15**) have demonstrated that the toxic effects produced in certain plants grown on acid soil are caused primarily by the presence of aluminum in the soil. Poor growth of the plants has been attributed to a phosphorus deficiency caused by the precipitation of phosphorus in the soil by aluminum (**3, 5, 6, 8, 10, 11, 12, 14**). On the other hand, some research (**4, 13, 17**) has indicated that aluminum may precipitate phosphorus within the plant, and thus make it unavailable for metabolic processes.

As recorded in an earlier publication (**20**), the writer grew barley in drip culture solutions wherein the root system was divided, each half being placed in separate containers, and receiving different culture solutions. By this method a plant could have access both to aluminum and to phosphorus, and precipitation in the culture solution could be avoided. Analyses for phosphorus and for aluminum in the various plant fractions revealed that the internal precipitation of phosphorus by aluminum evidently plays an important rôle in the poor development of plants grown in contact with aluminum. The findings reported in this paper further support the thesis that aluminum toxicity may in part be caused by the precipitation of phosphorus internally, thus inactivating phosphorus.

Procedure

Barley seeds were germinated on a disc in distilled water. One week later the plants were transferred to a modified HARTWELL and PEMBER (**8**) culture solution. The modification consisted in increasing the phosphorus to 12 p.p.m., and in adding 1 p.p.m. each of boron and manganese. Two hundred plants were placed in this solution. Three hundred plants were placed in a similar solution to which had been added 8 p.p.m. of aluminum.

TABLE I

PHOSPHORUS CONTENT OF BARLEY WITH VARIOUS EXTRACTING MEDIA

DETERMINATIONS	NORMAL PLANTS			ALUMINUM-TOXIC PLANTS		
	TOPS	ROOTS	WHOLE	TOPS	ROOTS	WHOLE
Dry weight, grams	83.34	16.66	100.00	18.50	8.35	26.85
Total phosphorus, %	0.86	1.07	0.89	0.76	2.30	1.24
Water-soluble phosphorus, %	0.82	0.92	0.84	0.70	0.39	0.60
pH 3 Sulphuric-acid-soluble phosphorus, %	0.87	1.07	0.90	0.75	0.53	0.68
pH 1 Sulphuric-acid-(1%) soluble phosphorus, %	0.84	1.08	0.88	0.80	2.02	1.18

[1] Contribution no. 642 of the Rhode Island Agricultural Experiment Station.

Five weeks later the plants were harvested, dried, and then ground in preparation for analysis.

Total phosphorus, and one per cent. sulphuric-acid-soluble phosphorus were determined by the method described by BERTRAMSON (1). To determine water-soluble phosphorus, and pH 3 sulphuric-acid-soluble phosphorus, a 0.5-gram sample of plant material in 50 ml. of the extracting medium was placed on a mechanical shaker for thirty minutes, filtered, and the amount of phosphorus in the filtrate ascertained by the method of TRUOG and MEYER (19), using a photoelectric colorimeter. The results are recorded in table I.

Discussion

The toxicity of aluminum is demonstrated by the low yield of the plants grown in the culture solution containing aluminum, a reduction amounting to 73.15 per cent.

The percentage of total phosphorus in the aluminum-toxic plants is greater than that of the normal plants. An accumulation of phosphorus in these poorly developed plants indicates that some of the phosphorus is not being used in the necessary metabolic processes. By comparison with the amount of phosphorus in the normal plants there was sufficient phosphorus in the aluminum-toxic plants to produce a larger yield than that obtained.

Attention is called to the distribution of the phosphorus in the plants grown in the culture solution containing aluminum. Although there is about 50 per cent. reduction in the yield of the roots as compared with the normal plants, the amount of phosphorus in the former is 2.3 per cent., in contrast with 1.07 per cent. in the normal roots. This would indicate that a good portion of the phosphorus is precipitated in the root system, and hence becomes unavailable for the growth of the plants.

The data reveal that the water-soluble phosphorus in the low-yield aluminum plants is 0.6 per cent., as compared with 1.24 per cent. of total phosphorus. With a total phosphorus content of 2.3 per cent. in the roots of these plants, better growth could be expected. With but 0.39 per cent. of water-soluble phosphorus present, it is evident, however, that most of the phosphorus is in a form which cannot be translocated to the growing regions of the plant in amounts sufficient for vigorous growth. PIERRE and STUART (17) found that the plant sap from Cos lettuce grown with aluminum in the culture solution contained only from one-fifth to one-seventh as much soluble inorganic phosphorus as those not receiving aluminum. This, in conjunction with other data, led them to believe that aluminum precipitates phosphorus within the plant making it unavailable for utilization by the plant.

If phosphorus is precipitated in the plant as aluminum phosphate, a method which would distinguish between organically and inorganically bound phosphorus should show relatively high amounts of inorganically bound phosphorus in the aluminum plants as compared with the normal plants. Such a method has been described by BERTRAMSON (1). Inor-

ganically bound phosphorus is obtained by extracting with one per cent. sulphuric acid solution. Organically bound phosphorus is determined by the difference between the inorganic fraction and total phosphorus. The results recorded in the table are typical of numerous determinations which were obtained by the writer with this method. In most instances the analyses for total phosphorus and for inorganic phosphorus were very similar. Whatever differences were obtained were hardly significant, nor did the results show a consistent trend. Organic phosphorus was a small negative quantity as often as a small positive quantity.

In order to find an extracting medium which might differentiate between the phosphorus combined with aluminum, and phosphorus otherwise bound, a series of sulphuric acid solutions varying by units of 0.5 pH from pH 1 to pH 5 was prepared. A 0.5-gram sample of plant material from the tops of the normal barley plants in 50 ml. of each of the nine solutions of acid served as checks against nine similar solutions to which aluminum phosphate as $AlPO_4$ in one group, and as $Al_2(HPO_4)_3$ in another group had been added to the plant material. The solution which just failed to extract phosphate from either of the salts was that with a hydrogen ion concentration of pH 3. PATTEN (**16**) stated that aluminum phosphate in hydrochloric acid was precipitated between pH 3 and pH 3.5. MAGISTAD's (**10**) data show that at pH 5 there was an amount of aluminum equivalent to 2 p.p.m. of aluminum oxide in solution, and that there was a rather rapid increase in solubility as the acidity increased beyond pH 4.5. LINE (**9**) found that aluminum was precipitated as the phosphate from a solution of its salts between pH 3 and pH 4. In extracting phosphorus from soil TRUOG (**18**) used a sulphuric acid solution buffered with ammonium sulphate at pH 3. This solution extracted "readily available" phosphorus, and did not extract "difficultly available" phosphorus. The latter was considered to exist largely as basic ferric phosphate, and possibly also as aluminum and titanium phosphates. These experiments indicate that an extraction solution of sulphuric acid of pH 3 could be used to determine whether or not there was a difference in the aluminum phosphate content of the barley plants grown in the culture solutions used.

As recorded in table I the amounts of phosphorus extracted from the normal plants by the pH 3 acid are practically identical with the total phosphorus content. In the poorly developed plants grown in the culture solution containing aluminum, however, the amount of phosphorus in the whole plant extracted by the acid is 0.68 per cent., whereas the total phosphorus is 1.24 per cent. The comparison is still more striking when the amount of acid-extracted phosphorus of the roots, 0.53 per cent., is compared with the total phosphorus content of the roots, 2.3 per cent. From this evidence, coupled with the fact that all of the phosphorus was soluble in the sulphuric acid of pH 1 which dissolves aluminum phosphate, it is assumed that the phosphorus which the pH 3 acid failed to extract is combined with aluminum. The inactivation of phosphorus by aluminum principally in the roots

thus creates an actual deficiency of phosphorus in the various meristematic regions of the plant with resultant poor growth.

Summary

Analyses of barley plants grown in culture solutions with and without aluminum indicate that there is a greater percentage of phosphorus in the aluminum-toxic plants than in the normal plants. This accumulation of phosphorus is particularly marked in the roots. A low percentage of water-soluble phosphorus in these plants indicates that the phosphorus is inactivated.

A sulphuric acid solution of pH 3 extracted practically all of the phosphorus from the normal plants, but extracted much smaller quantities from the plants grown in contact with aluminum. The high total phosphorus and low water-soluble phosphorus content of the latter was attributed to the precipitation of phosphorus by aluminum within the plant. This mutual precipitation occurs primarily in the roots, and thus causes a phosphorus deficiency in meristematic regions which is reflected in sharp reductions in yield.

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BRIEF PAPERS

PYRROLE DERIVATIVES AND IRON CHLOROSIS IN PLANTS

S. Aronoff and G. Mackinney

Pollacci and Oddo (**6**) have stated that alpha substituted pyrroles, such as the aldehyde and carboxylic acid may replace iron functionally in chlorophyll-deficient plants. As a corollary, it was believed that the function of iron in green plants was concerned with the formation of pyrrole derivatives possessing a labile alpha substituent. Other authors (**2, 3**) have not confirmed these experiments. The work was then repeated and the original results re-affirmed (**5, 7**). Following the negative work of Demidenko (**1**) the experiments were again repeated by Lodoletti (**4**) who extended the theory to include non-homologous water insoluble pyrrolic derivatives such as acetylmethylindole and acetylmethylbenzo-pyrrole. In view of the contradictory reports, we have repeated some of the work, and tested additional compounds. These included pyrrole α-aldehyde, pyrrole α-carboxylic acid, disodium bilirubinate, mesoporphyrin IX, succinimide, and tryptophane. All the compounds were tested with corn, and in addition, the aldehyde was tested with barley and Chlorella.

The data reported here are limited by the low yields obtained in the laboratory synthesis of the pyrrole derivatives. These must be carefully purified to ensure that the observed effect is not ascribed to an accompanying impurity which has been inadvertently added. Furthermore, the results were so clear-cut, as to the toxicity or harmlessness of the compounds tested, that this brief note would seem warranted.

Experimentation

Golden Bantam corn, treated with 1 per cent. $HgCl_2$ and then 70 per cent. alcohol was germinated in the dark at 30° C. for 4 to 5 days, exposed to light for 2 to 3 days, and then transferred to aerated nutrient solutions (**8**) containing the essential elements except for iron. The time for almost complete chlorosis in the new leaves ranged from 5 days in the summer to 10 days in the winter. Sacramento barley was similarly germinated, and chlorosis was marked in from 4 to 7 days. *Chlorella vulgaris* was grown in a solution containing 1.5 per cent. glucose (purified by autoclaving with $CaCO_3$), 0.001 M KNO_3, K_2HPO_4, and $MgSO_4$. An air stream containing 5 per cent. CO_2 was passed through the culture. New cultures were grown as needed from 3 ml. of a 10-day-old suspension. Illumination was by means of 30-watt fluorescent lamps, 3 to 4 inches from the culture flasks.

The compounds tested were added in concentrations ranging from 0.025 to 0.250 gm. per liter. This dosage is from two-thirds to thirteen times the theoretical needed for synthesis of chlorophyll in the green controls.

Chlorophyll was estimated spectrophotometrically on acetone extracts of about sixty samples of homologous leaves in the different experiments, and the results for four of the compounds are summarized in table I.

In the case of the pyrrole aldehyde and carboxylic acid, the plants wilted, developing a purple color in the roots and leaves, and the degree of damage increased with increase in concentration. The aldehyde was more toxic than the acid, in which ten days was an average survival time, whereas the plants were dead in from 1 to 4 days with the former. Mesoporphyrin was also toxic, the plants showing severe wilt in 2 days, and dying within 5 days. Chlorophyll therefore was not determined.

To the Chlorella cultures there were added 32, 64, and 128 mg. of pyrrole aldehyde per liter. Purple discoloration developed within 48 hours at the higher concentrations, and after 72 hours at the low level.

TABLE I

CHLOROPHYLL CONTENT OF PLANTS WITH ADDED DERIVATIVES OR SUBSTITUTES FOR IRON

COMPOUND TESTED AND CROP	CHLOROTIC CONTROL	NO IRON + COMPOUND	IRON + COMPOUND	IRON CONTROL	REMARKS
Pyrrole aldehyde					Highly
(Corn)	1.00	1.26 ± 0.29*	††	4.53	toxic
(Barley)	1.00	1.17 ± 0.05	1.80 ± 0.05	2.30	"
Succinimide					
(Corn)	1.00	0.90 ± 0.06		4.55	Non-toxic
Disodium bilirubinate					
(Corn)	1.00	1.00 ± 0.05	13.2 ± 0.1	35.0‡	"
Tryptophane					
(Corn)	1.00	1.00 ± 0.06		35.0	"

* The high value is due to the wilted moribund condition of the samples.

† The toxicity of the aldehyde was apparent even in the presence of four times the necessary amount of iron.

‡ The controls have high values as the non-toxic compounds could be tested over longer periods of time.

No bilirubinate was visibly absorbed by the roots. Disodium bilirubinate is normally oxidized to biliverdinate in 12 hours. This did not happen in the culture solution in 8 days.

The tryptophane and succinimide were Eastman Kodak (techn.). Tryptophane was used without further purification. Succinimide was twice recrystallized from acetone. The mesoporphyrin was prepared from hemin by reduction of hemin with HI and red phosphorus, and recrystallized from HCl. Bilirubin was extracted from gall stones of cattle, and was carefully purified.

The pyrrole carboxylic acid was prepared from pyrrole by the action of CO_2 on the Grignard pyrrole. It was recrystallized in H_2O.

The aldehyde was synthesized from pyrrole by the usual REIMER-THIMANN method ($KOH + CHCl_3$). It was twice recrystallized from petroleum ether.

Summary

The theory that pyrrole derivatives may replace iron in the synthesis of chlorophyll by green plants has not been confirmed.

DIVISION OF FRUIT PRODUCTS
THE UNIVERSITY OF CALIFORNIA

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LENGTHENING THE STORAGE LIFE OF APPLES BY REMOVAL OF VOLATILE MATERIALS FROM THE STORAGE ATMOSPHERE

F. W. Southwick and R. M. Smock

McIntosh apples held in controlled atmosphere storage (*i.e.*, controlled oxygen and carbon dioxide levels) will keep two to three times as long as similar apples held in ordinary cold storage. When this technique is used, volatiles produced by the apples tend to accumulate in the gas tight chamber. Because of such accumulations the apple scald disease is likely to be severe unless shredded oiled paper is used in quantities around the fruit.

Attempts have been made to find a cheaper and more satisfactory control of this disease than the use of oiled papers. In an earlier report[1] it was noted that some promise was shown by various methods of removal of these volatiles by "air conditioning" the atmosphere. During the 1942–1943 season a semi-commercial trial with 1800 bushels of McIntosh apples was made of air conditioning the storage atmosphere with a highly refined oil. This technique controlled the scald on the more mature lots of fruit in storage; but on the greener lots there was four per cent. of scald. While this might have been considered commercial control, it is not perfect control. A semi-commercial trial of air conditioning the atmosphere of a 300-bushel controlled-atmosphere chamber full of McIntosh with activated lignite charcoal did not give commercial control of scald. While it controlled the disease on the mature lots of fruit, there was thirty per cent. of scald on the greener fruits.

In a series of trials with two-bushel lots of McIntosh apples in controlled-atmosphere storage, the only air conditioning agent that gave perfect control of scald was activated charcoal on which bromine had been adsorbed. Untreated activated charcoal did not give control. An oil wash for the atmosphere gave results comparable to those described in the semi-commercial test. Alkaline potassium permanganate did not give control.

A second result noted in the use of brominated charcoal was that the storage life of the apples was considerably lengthened (aside from scald control). Approximately two or three months was added to their storage life. Apparently enough ethylene is generated even in controlled atmosphere storage by the fruit, that its removal may retard ripening.

In laboratory trials it had been found that brominated charcoal was the only really effective agent in removing naturally evolved ethylene from rather ripe fruits. The effect of a rather ripe lot of apples in storage in stimulating the ripening of another less ripe lot of fruit in air storage (presumably through its evolution of ethylene) was eliminated by air conditioning the air with brominated charcoal.

It was found that activated lignite charcoal was not effective in controlling scald, although it seemed an excellent absorbent for artificially

[1] Smock, R. M., and Southwick, F. W. Some factors affecting the apple scald disease. Science n.s. **95**: 576–577. 1942.

added esters. This would seem suggestive that esters were not the causative agent of scald. The fact that bromine is so effective in controlling scald suggests that scald is in some way related to an unsaturated hydrocarbon.

The production of volatiles by McIntosh apples was less in 1942–1943 than in the previous season—so the results reported may not be as favorable in a year of high volatile production and high incidence of scald.

Preliminary experiments indicate that it is unlikely that scald can be easily controlled on a very scald-susceptible variety like Rhode Island Greening by air conditioning the atmosphere with brominated charcoal, since excessively rapid air movement is necessary to accomplish control even by ventilation with fresh air.

DEPARTMENT OF POMOLOGY
CORNELL UNIVERSITY

NOTES

Annual Election.—In the annual election of 1943, Dr. JOHN W. SHIVE was elected as a member of the executive committee for a term of three years. A tie vote for a member of the editorial committee has been resolved by the executive committee in favor of Dr. E. J. KRAUS, who has been elected for a term of three years.

Life Membership Committee.—The CHARLES REID BARNES life membership committee has been appointed by the president, Dr. BERNARD S. MEYER, of Ohio State University. The chairman of the committee is Dr. R. B. HARVEY, Minnesota. The other members of the committee are Dr. R. E. GIRTON, Purdue; Dr. F. S. HOWLETT, Ohio State University; Dr. G. S. AVERY, Connecticut College; and Dr. F. WENT, California Institute of Technology. The 1943 award is the 20th, and is to be awarded to a distinguished plant physiologist from some foreign land, according to the provision of the by-laws that every fifth award is to be made outside the United States. The task of making the selection is always a pleasant one, but made more difficult than usual by war conditions. Announcement of the award may be expected in the January, 1944, issue of PLANT PHYSIOLOGY. The original announcements have always been a part of the annual dinner ceremonies, but there is at the present moment no prospect of a national annual meeting. If, however, any regional meeting of the A.A.A.S. or other organizations are arranged, notices of such meetings will be made public and all members informed.

Purdue Section.—The Purdue section of the American Society of Plant Physiologists maintains its activities with an interesting series of programs. The section meets twice each month in Room 103 Coulter Hall, at 4:00 P.M. The following titles are on the program for the first half of the current season:

October 18.—L. J. SWIFT: The viscosimetric determination of free menthol and peppermint oil.

November 15.—G. B. CUMMINS: Some defoliation studies on tomatoes.

December 6.—W. R. MULLISON: Some recent experiments in plant nutrition.

December 20.—C. L. PORTER: Penicillin to date.

January 3, 1944.—G. O. MOTT: The association of grasses and legumes in pasture mixtures.

January 17.—E. W. STARK: Comparative anatomy of woods.

Dr. LAURENZ GREENE, Department of Horticulture, is chairman of the Purdue section, and represents the Purdue section on the executive committee of the society.

New England Section.—The executive committee of the New England section voted last spring that it was not advisable to hold the regular meeting. It was also decided that the organization should remain *in statu quo* with regard to officers until officers could be elected at a meeting to be held under more normal conditions. The secretary, Dr. LINUS H. JONES, suggested that members prepare papers, since it seemed that graduate students would not be available.

Committee on the Editorship.—The approaching retirement from active service of the present editor-in-chief of PLANT PHYSIOLOGY has made it necessary to consider the appointment of a successor as of the end of 1944. To meet this situation, Dr. LOOMIS, during his term as president, named a committee to canvass the situation. The personnel of the committee is as follows: Chairman, Dr. O. F. CURTIS, Cornell University; Dr. F. P. CULLINAN, the U. S. Department of Agriculture; Dr. B. M. DUGGAR, the University of Wisconsin; Dr. GEORGE W. SCARTH, McGill University; and Dr. SAM TRELEASE, Columbia University.

The executive committee will, of course, have to make the final decision. In December, 1943, it will be two years since this matter first came up, and a prompt decision is desirable in order that the incoming editor-in-chief may become familiar with every phase of the editorial work during the remaining year in which cooperation will be possible. The cancellation of the annual meeting in 1942 prevented the usual meeting of the executive committee, and no opportunity for discussion of the problem has arisen. The editor-in-chief desires to cooperate with those who have the responsibility of making the selection, and will be happy to correspond with any members of either committee. Time is of the essence, and there is little time to lose.

Errata.—Attention of members and subscribers is called to the list of errata at the close of the table of contents of volume 18. We regret the errors, and suggest that owners of volumes enter the corrections so that at no future time could the errors cause other errors to be made. Thanks are accorded to those who kindly called attention to the mistakes. All of our authors are requested to take the same interest in their own papers, and to report to the editorial office anything that needs correction.

George Lincoln Teller.—In the death of GEORGE LINCOLN TELLER on November 13, 1942, the American Society of Plant Physiologists lost one of its older members. Mr. TELLER was a well-known figure in cereal chemistry and research. Since 1926 he had been president of the Columbus Laboratory in Chicago.

He was born at Colon, Michigan, January 23, 1867. His education was obtained in the public schools of Michigan, and at the Michigan State College, which granted him the B.S. degree in 1888, and M.S. in 1893. For two

years, 1888–1890, he was assistant chemist at the Experiment Station at East Lansing. In 1890 he was called to the Arkansas Station as chemist, a position which he held for nine years. He was connected with the Chidlow Institute in Chicago for several years, beginning in 1899; and in 1902 he began work with the Columbus Laboratory. In 1926 he began his term as president of the laboratory.

He was deeply interested in the chemistry of flour and other cereal products, and studied the proteins, mineral content, sugars, diastatic activity, etc. He was also a student of starch grain formation and structure. Two of his numerous papers were published in PLANT PHYSIOLOGY: one on changes in nitrogen compounds in the wheat grain at different stages of development, in 1935; the other on plant diastase in evidence as to the formation and structure of the starch granules, in 1938. In the Journal of Biological Chemistry for 1936 we note a paper entitled: Evidence concerning two types of diastase. He contributed a number of papers to Cereal Chemistry, and a series of bulletins from the Columbus Laboratory, reprinted from the Northwestern Miller, National Miller, American Food Journal, etc.

He was a man of dynamic personality, and contributed to the growth and development of the Columbus Laboratory into a fine testing and research organization. His contribution was a worthy one, a definite humanitarian service. He will be greatly missed by his colleagues and friends.

Mineral Deficiencies.—A very useful color atlas and guide to mineral deficiencies in plants has been published by the University of Bristol Agricultural and Horticultural Research Station at Long Ashton, Bristol. The work has been prepared by T. WALLACE, under the title: *The Diagnosis of Mineral Deficiencies in Plants by Visual Symptoms.*

There are five chapters of text, following the preface and introduction, titled as follows: Essential points in the nutrition of plants; soils in relation to the supply of mineral nutrients; methods of determining mineral deficiencies in crops; visual symptoms of deficiencies in crops; and use of the visual method of diagnosis in the field. The discussion is concise and yet adequate. The most important part of the work, of course, is the atlas of colored plates which are printed two to the page, but they are large enough to convey to the student the essential symptoms of deficiency. The total number of plates is 114, a very fine coverage. They are arranged into a number of groups: 1. Potatoes grown in sand cultures. 2. Natural autumn foliage tints. 3. Deficiencies as shown in field crops. The deficiencies illustrated are: phosphorus, calcium, magnesium, potassium, iron, manganese, and boron. 4. Symptoms which may be confused with natural deficiencies. These include virus injuries, insect damage, and chloride toxicity.

There are always difficulties in reproduction of colors for such works, and in this case the principal difficulty seems to be in shades of green, which seem to the reviewer to be somewhat too blue in some instances. On the

whole, however, it is a very valuable guide, and should prove extremely serviceable to the practical grower, as well as to the student who is trying to become familiar with troubles of deficiency at sight.

Many American laboratories and individual workers will want this valuable atlas, both for its text, and for its colored plates. The price is only 10/– net, and the orders should be addressed to H.M. Stationery Office, York House, Kingsway, London W.C.2.

Soil-Yield Theory.—This work, in German, discusses the problems of yield from the standpoint of mathmatical computation. It is entitled *Bodenertrags-Theorie,* and the author is Dr. KARL DÜRR. The introduction deals with the methods, and fundamentals of yield theory. The first chapter presents the scientific-technical considerations, plant physiological, and the application of yield laws to agriculture. The second chapter, with eleven sections, deals with the entire course of yield or production as an agricultural problem, and the economics of agricultural production. It takes up and discusses in thoroughly modern fashion the problems that confronted MALTHUS and JOHN STUART MILL . . . and the problems of gross receipts (Rohertrag), net receipts (Reinertrag), proceeds (Erlös), and expenses (Aufwand). The presentation necessarily involves mathematical equations, but these are readily understood by anyone who has given the matter some attention.

The book gives a very good summary of land yield and rental problems, based upon sound scientific principles. Eight text figures assist one in grasping the mathematical relations. It ought to be a valuable work for technical agronomists and plant physiologists, but would have to be interpreted in simple terms if it is to become available to the ordinary agriculturist. Agricultural economy is a complex problem, and few dirt farmers have any conception of the intricate economic relationships of production factors.

The book, with brief index, occupies only 96 pages. It is bound in paper. The publisher is Verlag Fr. Dürig, Ostermundigen-Bern, Switzerland. The price quoted is very reasonable, 4.5 Swiss francs. Orders should be sent to the publisher at the address given.

Cellulose and Cellulose Derivatives.—A compendious work dealing with cellulose and its derivatives has been prepared by a group of specialists under the editorship of Dr. EMIL OTT, Director of Research of the Hercules Powder Co., Wilmington, Delaware. It belongs in the series dealing with High Polymers, volume V in the series. There are several dozen contributors to the volume, and the contents are arranged into ten chapters with the following titles: Occurrence of cellulose; chemical nature of cellulose and its derivatives; structure and properties of cellulose fibers; carbohydrates normally associated with cellulose in nature; lignin and other noncarbohydrates; preparation of cellulose from its natural sources; bleaching

and purification of cellulose; derivatives of cellulose; physical properties of cellulose and its derivatives; and technical applications of the physical properties of cellulose and its derivatives.

The indexes are unusually full, and occupy 66 pages. The entire monograph contains 1176 pages, and its size of course makes it an expensive book to own. The price quoted is $15.00 per copy. Nevertheless, one cannot anywhere else obtain so full an account of cellulose, its nature and uses. The Interscience Publishers, Inc., deserve congratulations for the fine service they are rendering to scientific and industrial progress in the publishing of such books as *Cellulose and Cellulose Derivatives.*

Copies may be ordered from the publishers, Interscience Publishers, Inc., 215 Fourth Ave., New York, N. Y.

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[1] Papers published in PLANT PHYSIOLOGY are indexed in The Agricultural Index, The W. H. WILSON Co., New York.

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AMERICAN SOCIETY

OF

PLANT PHYSIOLOGISTS

Bulletin No. 14

Compiled by
PAUL J. KRAMER, Secretary-Treasurer
Durham, N. C., June 1, 1943

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SPECIAL AWARDS AND HONORS

THE STEPHEN HALES PRIZE AWARDS

1929—Dennis Robert Hoagland of the University of California, for his work in plant nutrition.

1930—Wightman Wells Garner of the United States Department of Agriculture, for his development of the concepts of photo-periodism.

1932—Hubert Bradford Vickery of the Connecticut Agricultural Experiment Station for his contributions to plant-protein chemistry.

1934—Charles Albert Shull of the University of Chicago, for distinguished service to the science of plant physiology.

1936—Kenneth Vivian Thimann of Harvard University, for his work with plant-growth hormones.

1938—John Wesley Shive of the New Jersey Agricultural Experiment Station, for his contributions to plant nutrition.

1940—Philip Rodney White of the Rockefeller Institute for Medical Research, for his pioneer work and major contributions to plant tissue culture.

1942—Cornelis Bernardus van Niel of Leland Stanford University, for his work in microbiology and photosynthesis.

CHARLES REID BARNES LIFE MEMBERSHIP AWARDS

1926—Burton Edward Livingston, Johns Hopkins University.

1927—Francis Ernest Lloyd, McGill University.

1928—Herman Augustus Spoehr, Carnegie Institution.

1929—Charles Albert Shull, University of Chicago, and George James Peirce, Stanford University.

1930—Rodney Howard True, University of Pennsylvania; deceased 1940.

1931—Charles Orville Appleman, University of Maryland.

1932—Charles Frederick Hottes, University of Illinois.

1933—James Bertram Overton, University of Wisconsin; deceased 1937.

1934—Frederick Frost Blackman, Cambridge University.

1935—Frank Marion Andrews, University of Indiana; deceased 1940.

1936—Daniel Trembly MacDougal, Carnegie Institution.

1937—Alexander Pierce Anderson, Red Wing, Minn., deceased 1943, and Homer LeRoy Shantz, U. S. Department of Agriculture.

1938—Ludwig Yost, Heidelberg University.

1939—Winthrop J. V. Osterhout, Rockefeller Institute.

1940—William Francis Ganong, Smith College; deceased 1941.

1941—Benjamin Minge Duggar, University of Wisconsin.

1942—Olenus Lee Sponsler, University of California at Los Angeles.

CORRESPONDING MEMBERS OF THE SOCIETY

1932—Frederick Frost Blackman, Cambridge University.

1932—F. A. F. C. Went, University of Utrecht; deceased 1935.

1934—Gottlieb Haberlandt, University of Berlin.

1934—V. N. Lubimenko, Leningrad; deceased 1937.

1935—Nicolai A. Maximov, Moskva.

1935—Hans Molisch, University of Wien; deceased 1937.

1935—Bohumil Nemec, Charles University, Praha.

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1937—Alfred Ursprung, University of Fribourg.

1938—Sir John Russell, Rothamsted Experiment Station.

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Abersold, John N., Chemist-Physiologist, American Smelting and Refining Co., 700 McCormick Bldg., Salt Lake City, Utah. Light; sulfur dioxide injury.

Adams, W. R., Assoc. Prof. Botany and Forestry, Univ. of Vermont, Burlington, Vt. Silvics and forest ecology.

Addicott, Frederick T., Instructor, Santa Barbara State College, Santa Barbara, Calif. Plant hormones; cell division.

Addoms, Ruth M., Assoc. Prof. Botany, Duke Univ., Durham, N. C. Mineral nutrition; physiological anatomy.

Akamine, Ernest K., Asst. in Plant Physiology, Hawaii Agri. Expt. Sta., Honolulu, T. H. Seed germination.

Aldrich, Willard W., Senior Horticulturist, Bureau of Plant Industry Station, Beltsville, Md. Water relations of fruit trees; tree root behavior.

Allison, Franklin E., Senior Chemist, Bureau of Plant Industry Station, Beltsville, Md. Biochemical nitrogen fixation; soils and fertilizers.

Altschul, Aaron M., Associate Chemist, Southern Regional Research Laboratory, New Orleans, La.

Anderson, Donald B., Prof. Botany, North Carolina State College, Raleigh, N. C. Plant cell walls.

Anthony, R. D., Prof. Pomology, Pennsylvania State College, State College, Pa. Fruit tree nutrition; fruit tree understocks; storage.

*Appleman, C. O., Prof. Plant Physiology and Dean of the Graduate School, Univ. of Maryland, College Park, Md. Plant physiology and biochemistry.

Appleman, David, Asst. Prof. Plant Nutrition, Univ. of California, Los Angeles, Calif. Chlorophyll formation; chlorosis.

Arens, Karl, Prof. of the University of Brazil, Rua Marques de Sao Vicente No. 29, Casa 7-Gavea, Rio de Janeiro, Brazil. Photosynthesis; mineral metabolism; Peronosporaceae.

Armstrong, Geo. M., Head, Dept. of Botany and Bacteriology, Clemson College, Clemson, S. C. Nutrition of cotton; cotton fibers.

Arndt, C. H., Assoc. Botanist, S. C. Agri. Expt. Station, Clemson, S. C. Water relations of plants; physiology of cotton seedlings.

Arnon, D. I., Assistant Professor of Plant Nutrition and Assistant Plant Physiologist, 3048 Life Science Bldg., Univ. of California, Berkeley, Calif. Mineral nutrition; micronutrients.

Aslander, Alfred, Kungl Tekn. Hogskolan, Drottninggatan 95 A, Stockholm, Sweden.

Atwood, W. M., Prof. Plant Physiology, Oregon State College, Corvallis, Ore. Germination; respiration.

Auchter, E. C., Agricultural Research Administration, U.S.D.A., Washington, D. C. Physiology of crop plants.

Avery, G. S., Jr., Prof. Botany and Director of the Connecticut Arboretum, Connecticut College, New London, Conn. Plant hormones; growth and development.

Babb, M. F., Assoc. Physiologist, U.S.D.A., Cheyenne Hort. Field. Sta., Cheyenne, Wyo. Physiology of vegetable crops.

Bacon, Franklin J., Prof. Botany and Pharmacognosy, Western Reserve Univ., Cleveland, Ohio. Glucosides; volatile oil metabolism.

Bailey, I. W., Prof. Plant Anatomy, Biological Laboratories, Harvard Univ., Cambridge, Mass. Cambium and its derivative tissues; the plant cell wall.

Bailey, Lowell F. Plant Physiologist, Dept. of Chemistry, Agricultural Experiment Station, Lexington, Ky.

Bailey, Wallace K., Georgia Expt. Sta., Experiment, Ga.

Bailey, W. M., Head, Dept. of Botany, Southern Ill. State Normal Univ., Carbondale, Ill.

Bair, Roy A. Everglades Experiment Station, Belle Glade, Fla. Weather and crop-yield relationships.

Bakke, Arthur L., Res. Prof. Plant Physiology, Iowa Agric. Expt. Sta.; Agent U.S.D.A., Ames, Iowa. Physiology; herbicides; storage.

Baldwin, I. L., Prof. Agricultural Bacteriology, Univ. of Wisconsin, Madison, Wis. Physiology of micro-organisms.

Ball, Charles D., Jr., Assoc. Prof. Biochemistry, Mich. State College, East Lansing, Mich. Wheat oil; anti-oxidants; protein precipitation.

Banfield, Walter M., Haddon Hall, Atlantic City, N. J.

Barr, C. Guinn, Dept. of Botany, Mich. State College, East Lansing, Mich. Plant chemistry; spectographic analysis; respiration.

Bartholomew, Elbert T., Plant Physiologist, Citrus Expt. Sta., Riverside, Calif. Citrus.

Barton, A. Willis, Head Biology Dept., Kansas State College, Hays, Kans. Enzymes; hydrogent ion concentration.

Bean, Ross S., 2335 Spaulding Ave., Berkeley, Calif. Mineral nutrition; nitrogen metabolism.

Bear, Firman E., Dept. of Soils, Agri. Expt. Sta., New Brunswick, N. J. Fertilizers; nutrition.

Beath, Orville A., Prof. Chemistry and Research Chemist, Univ. of Wyoming, Laramie, Wyo. Micronutrients; poisonous plants.

Beauchamp, C. E., Manzana De Gomez 555, Havana, Cuba. Fertilizers and plant nutrition.

Beaumont, J. H., Dir. Hawaii Agri. Expt. Sta., Honolulu, T. H. Flowering and fruiting of mango, coffee.

Beck, W. A., Prof. Botany, Univ. of Dayton, Dayton, Ohio. Osmotic quantities; growth promoting substances; plant pigments; growth.

Beckenback, J. R., Truck horticulturist in charge of Vegetable Crops Laboratory, Bradenton, Fla. Nutrition; physiological disorders of vegetable crops.

Beeskow, Herbert C., Asst. Prof. Plant Physiology, Dept. of Botany, Mich. State College, E. Lansing, Mich. Effects of light upon plants; germination; vitamin C; mineral nutrition.

Benedict, H. M., Guayule Research Project, P. O. Box 1708, Salinas, Calif. Physiology of forage grasses.

Bennett, Emmett, Goessman Laboratory, Massachusetts State College, Amherst, Mass.

Bennett, James P., Prof. Plant Physiology, 110 Hilgard Hall, Univ. of Calif., Berkeley, Calif. Plant nutrition.

Bennett, Ralph E., 1822 Eastern Parkway, Louisville, Ky.

Bergman, Herbert F., Senior Pathologist, Bur. of Plant Industry, U.S.D.A., Goessman Laboratory, Mass. State College, Amherst, Mass. Pathology and physiology of cranberries and blueberries.

Berry, L. Joe, Asst. Prof. Biology, Bryn Mawr Col., Bryn Mawr, Pa. Bioelectrics.

Biale, J. B., Instructor in Subtropical Horticulture, 405 Hilgard Ave., Los Angeles, Calif. Respiration and transpiration.

Bibbey, Robert O., Instructor in Field Husbandry, Univ. of Saskatchewan, Saskatoon, Sask, Can. Germination of weed seeds.

Biddulph, Orlin, Asst. Prof. Botany, State College of Washington, Pullman, Wash. Plant nutrition.

*†Blackman, F. F., Reader in Plant Physiology, Botany School of Cambridge, Cambridge, England. Respiration; photosynthesis.

Bonner, James, Asst. Prof. Plant Physiology, Calif. Inst. of Technology, Pasadena, Calif. Chemical physiology; plant growth factors.

Bourne, B. A., Chief, Agricultural Research, U. S. Sugar Corp., Clewiston, Fla. Pathology; physiology; agronomy; sugar cane genetics.

Brenchley, Winifred E., Rothamsted Experimental Station, Harpenden, Herts, England. Plant nutrition; minor elements; weeds.

Brierly, W. G., Prof. Horticulture, University Farm, St. Paul, Minn. Cold resistance of fruit plants.

Briggs, George S., 977 Center Avenue, Grandview Heights, Lancaster, Pa.

Brown, H. D., Prof. Vegetable Gardening, Hort. Dept., Ohio State Univ., Columbus, Ohio. Quality of vegetables; fertilizer tests; breeding; water culture.

Brown, J. G., Head, Dept. Plant Pathology, Univ. of Ariz., Plant Pathologist, Ariz. Expt. Sta., Box 4672, University Station, Tuscon, Ariz. Cotton diseases; bacterial diseases of plants.

Brown, James W., 1916 M St. N. E., Apt. 2, Washington, D. C. Respiration; after ripening; germination.

Brown, Russell G., Asst. Prof. Botany, Univ. of Maryland, College Park, Md. Ecology.

Broyer, Theodore C., Jr., Plant Physiologist, Div. of Plant Nutrition, Univ. of Calif., Berkeley, Calif. Plant nutrition; absorption and transport of solutes and water.

Buller, A. H. Reginald, Prof. Emeritus, Dept. of Botany, Univ. of Manitoba, Winnipeg, Canada. Fungi; physiological problems in general.

Burk, Dean, Senior Chemist, Nat'l Cancer Inst., U. S. Public Health Service, Bethesda, Md. Intermediate metabolism; nitrogen fixation; photosynthesis.

Burkhart, Leland, Assoc. Agronomist, North Carolina State College, Raleigh, N. C. Nutrition of agronomic and horticultural crops.

Burkholder, Paul R., Dept. of Botany, Yale Univ., New Haven, Conn. Plant growth; hormones.

Burns, G. Richard, Assoc. Prof., Hunter College, 695 Park Ave., New York City, N. Y. Photosynthesis.

Burr, Geo. O., Prof. Botany, Univ. of Minn., Minneapolis, Minn. Photosynthesis; light effects; lipids.

Burrell, R. C., Prof. Agric. Chem., Ohio State Univ., Columbus, Ohio. Plant chemistry.

Burrows, Lieut. Fred W., A.A.F., A.F.T.T.C., Sheppard Field, Wichita Falls, Texas.

Cailloux, Marcel, Institute Botanique, 4101 est Rue Sherbrooke, Montreal, Can.

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Caldis, P. D., California Packing Company, 240 Front St., San Francisco, Calif.

Carey, Cornelia Lee, Asst. Prof. Botany, Barnard College, Columbia University, New York, N. Y. Bacteriology.

Carolus, Robert L., Horticulturist, Va. Truck Expt. Sta., Norfok, Va. Mineral nutrition of vegetable crops.

Carr, R. H., Prof. Agric. Chemistry, Purdue Univ., Lafayette, Ind. Biochemistry.

Carrol, J. C., Asst. in Agronomy, Ohio Agri. Expt. Sta., Wooster, Ohio. Agrostology; temperature and moisture relations in plants.

Carter, Edward P., Assistant Plant Pathologist, U.S.D.A., Division of Cereal Crops and Diseases, Dept. of Botany, Oklahoma A. and M. College, Stillwater, Okla.

Casamajor, Robert, Pres. Pasadena Flower Show Assn., 385 East Green St., Pasadena, Calif.

Castaneda, Manuel A., Prof. Plant Physiology, Instituto Politecnico Nacional, Mexico, D. F., Apartado Postal-7016. Plant biochemistry; respiration; metabolism; mineral nutrition; soil biochemistry; phytohormones.

Castle, Edward S., Assoc. Prof. Physiology, Harvard Univ. Biol. Labs., Divinity Ave., Cambridge, Mass. Growth and structure of cell wall; physiology of fungi.

Caughey, Mary, Science Dept., East Carolina Teachers College, Greenville, N. C. Physiology of pocosin plants.

Chadwick, L. C., Assoc. Prof. Horticulture, Dept. of Horticulture, Ohio State Univ., Columbus, Ohio. Propagation and nutrition of ornamental plants.

Chandler, F. B., Assoc. Physiologist, Maine Agri. Expt. Sta., Orono, Maine. Plant nutrition.

Chandler, Ray C., Farallone Packing Co., 4635 E. 12th St., Oakland, Calif.

Chandler, W. H., Prof. Horticulture, Univ. of Calif., 405 Hilgard Ave., Los Angeles, Calif. Horticulture.

Chang, H. T., Graduate student, Department of Botany, Iowa State College, Ames, Iowa. Effects of environment on physiological processes.

Chapman, H. D., Assoc. Chemist, Univ. of California, Citrus Expt. Sta., Riverside, Calif. Citrus nutrition.

Charles, Brother H., St. Mary's College, Winona, Wis.

Cheney, Ralph H., Chairman Biology Dept. and Prof. of Biology, L. I. Univ., Brooklyn, N. Y. Plant alkaloids from the genus Coffea; effect of purine compounds and nicotine upon plants and animals.

Childers, Norman F., Asst. Prof. Horticulture, Ohio State Univ., Columbus, Ohio. Photosynthesis; transpiration; respiration; nutrition.

Clark, Daniel G., Asst. Prof. Botany, Cornell Univ., Ithaca, N. Y. Photosynthesis; mineral nutrition; hormones.

Clark, Harold E., Head, Physiology-Soils Dept., Pineapple Expt. Sta., Box 3166, Honolulu, T. H. Physiology of pineapple; nitrogen nutrition.

Clark, Norman A., Assoc. Prof. Soil Chemistry, Iowa State College, Ames, Iowa. Mineral nutrition; accessory growth-substances.

Clark, Orton L., Assoc. Prof. Botany, Mass. State College, Amherst, Mass. Light and growth; plant motility; ecology.

Clements, Frederick E., 190 Meradero Lane, Santa Barbara, Calif. Ecology.

Clements, Harry F., Prof. Botany and Plant Physiology in Expt. Sta., Univ. of Hawaii, Honolulu, T. H. Climate and plant growth; bud behavior; translocation.

Clum, Harold H., Assoc. Prof. Botany, Hunter College, P. O. Box 572, Chappagua, N. Y. Water relations.

Collander, Runar, Prof., Botanical Institute, Univ. of Helsingfors, Helsingfors, Finland. Cell permeability; mineral nutrition.

Conner, Herbert W., 2032 W. 110th Place, Morgan Park Sta., Chicago, Ill.

Conrad, Carl M., Southern Regional Research Laboratory, New Orleans, La. Chemical composition and utility of the cotton fiber.

Conrad, John P., Assoc. Prof. Agronomy, Div. of Agronomy, Davis, Calif. Effects of crops on soil and crops following.

Cooil, Bruce J., 20 Midway St., Salinas, Calif.

Cooke, Douglas A., Plant Physiologist, Hawaiian Sugar Planters' Assn., Honolulu, T. H. Mineral nutrition of sugar cane.

Cooley, J. S., Senior Pathologist, Bureau of Plant Industry Station, Beltsville, Md. Pathology.

Cooper, H. P., Dean of Agriculture and Director of the Agri. Expt. Sta., Clemson College, Clemson, S. C. Mineral nutrition; ecology.

Cooper, William C., U.S.D.A. Experiment Station, Mayaguez, Puerto Rico. Plant hormones.

Cosper, Lloyd C., Independent research, 1779 E. Mendocino St., Altadena, Calif. Orchid nutritional studies; effect of growth hormone on orchids; orchid seed germination.

Craft, James H., Jr., Dept. of Botany, State University of Iowa, Iowa City, Iowa. Growth, metabolism of succulents.

Crafts, Alden S., Assoc. Prof. Botany and Assoc. Bot. in the Expt. Sta., Botany Div., Univ. of Calif., Davis, Calif. Structure and function of phloem; chemical weed control.

Cruess, W., Prof., Division of Viticulture and Fruit Products, Univ. of Calif., Berkeley, Calif. Fermentation; enzymes.

Cullinan, F. P., Assistant Chief, Bureau of Plant Industry, Bureau of Plant Industry Station, Beltsville, Md. Nutrition and physiology of stone fruits.

Culpepper, Charles W., 4435 N. Pershing Drive, Arlington, Va. Fruit and vegetable preservation.

Currier, Herbert B., Research Chemist, Basic Vegetable Products Co., Vacaville, Calif. Water relations; cellular physiology.

Curtis, Otis F., Prof. Botany and Plant Physiology, Cornell Univ. Agri. Expt. Sta., Ithaca, New York. Translocation; water relations.

Curtis, Otis F., Jr., Instructor Floriculture, 405 Hilgard Ave., Los Angeles, Calif. Physiology of ornamental plants.

Daniel, T. W., Western Washington Experiment Station, Puyallup, Wash.

Danielson, Loran L., 500 Sixth St., Rochelle, Ill. Sex; plant nutrition; physiology of genetics.

Darbaker, Leasure K., Prof. Microbiology and Pharmacognosy, School of Pharmacy, Univ. Pittsburgh, Pittsburgh, Pa. Micro-biology.

Davidson, O. W., Asst. Prof. Pomology, Assoc. Biochemist in Hort., N. J. Agri. Expt. Sta., New Brunswick, N. J. Plant nutrition.

Davies, P. A., Prof. and Head, Dept. of Biology, College of Liberal Arts, Univ. of Louisville, Louisville, Ky. Physiology; biochemistry.

Davis, A. R., Prof. Plant Physiology, Univ. of California, Berkeley, Calif. Relation of light to growth.

Davis, Charles H., R.F.D. 2, Box 428, Glendale, Ariz. Moisture relations.

Davis, L. D., Assoc. Prof. Pomology, University Farm, Univ. of California, Davis, Calif.

Davis, M. B., Dominion Horticulturist, Central Expt. Farms, Ottawa, Ont., Can.

Davis, Ward B., Assoc. Chemist (Biological), U.S.D.A., Lab. of Fruit and Veg. Chemistry, 148 Mission Rd., Los Angeles, Calif. Enzymes; vitamins.

Dawson, Ray F., 324 Guyot Hall, Princeton University, Princeton, N. J.

Day, Dorothy, Plant Physiologist, California Central Fibre Corporation, Pisgah Forest, N. C.

Decker, Lieut. John P., Army Air Force, Station Hospital, Westover Field, Chicopee Falls, Mass.

DeLong, Walter Anthony, Asst. Prof. Chemistry, MacDonald College, Quebec, Can. Nutrition; respiration; methods of analysis.

Dennison, Raymond, 212 S. Poplar St., Oxford, Ohio.

DeRose, H. Robert, Asst. Chemist, Mass. State College, Amherst, Mass. Plant nutrition (effect of trace elements on the chemical composition of plants); plant biochemistry.

Deters, Merrill E., School of Forestry, Univ. of Idaho, Moscow, Idaho.

DeTurk, E. E., Prof. Soil Fertility, Univ. of Illinois, 306 Old Agri. Bldg., Urbana, Ill. Nutrition of crop plants; nutrient deficiency symptoms; strain and species variations in nutrient element requirements.

Dexter, Stephen Terry, Asst. Prof. and Res. Assoc., Farm Crops Dept., Mich. State College, East Lansing, Mich. Winter hardiness; physiology of weeds; forage crops.

Dimond, Albert E., Dept. of Botany, University of Nebraska, Lincoln, Neb. Physiology of fungi.

†Dixon, Henry H., Univ. Prof. Botany, Trinity College, Dublin, Ireland. Osmotic pressure; translocation.

Doneen, L. D., Junior Irrigation Agronomist, Division of Irrigation Investigations and Practice, College of Agri., Univ. of Calif., Davis, Calif. Plant-soil-water relations.

Doty, Hiram S., Prof. Biology, Simpson College, Indianola, Iowa. Ecology; physiology.

Downie, Andrew A., 295 Maple St., New Britain, Conn.

DuBuy, H. G., Asst. Prof. Plant Physiology, Univ. of Md., College Park, Md. General physiology; growth factors.

*Duggar, Benjamin M., Prof. Physiological and Applied Botany, Univ. of Wisconsin, Madison, Wis. Physiology; physiological pathology.

Dungan, Geo. H., Prof. Crop Production, Agronomy Dept., Univ. of Ill., Urbana, Ill. Adaption of corn, wheats, oats and barley; crop physiology.

Dunlap, A. A., Chief, Div. Plant Pathology and Physiology, Texas Agri. Expt. Sta., College Station, Texas. Physiological aspects of plant diseases; fruiting of cotton.

Dunn, Stuart, Asst. Prof. Botany and Asst. Botanist, Expt. Sta., Univ. of New Hampshire, Durham, N. H. Influence of soil textures and aeration on plant growth; plant propagation.

Dustman, Robert B., Head, Dept. Agri. Chem., Univ. of West Virginia, Morgantown, W. Va. Plant nutrition.

Dyer, Hubert J., Department of Botany, University of Chicago, Chicago, Ill. Lipid metabolism.

Eastwood, Thomas M., 851 Lincoln Way, Mishawaka, Ind. Plant nutrition; plant pathology.

Eaton, Frank M., Senior Plant Physiologist, U.S.D.A., College Station, Texas. Physiology of the cotton plant; salt, boron and water relations.

Eaton, Scott V., Asst. Prof. Botany, Univ. of Chicago, Chicago, Ill. Mineral nutrition; plant chemistry.

Eisenmenger, Walter S., Res. Prof. and Head Dept. of Agronomy, Mass. State College, Amherst, Mass. Antagonism; colloidal chemistry of soil.

Eleizalde, Luis M. de, Professor de Edafologia y Fisiologici Vegetal, Instituto Experimental de Agricultura. El Valle D. F. Venezuela. Plant nutrition and transpiration.

Eltinge, Ethel T., Asst. Prof. Botany, Mt. Holyoke College, S. Hadley, Mass. Mineral nutrition.

Emmert, E. M., Asst. Prof. Horticulture, Univ. of Ky., Lexington, Ky. Use of rapid tissue tests in determining the nutritive needs of plants.

Engard, Charles J., Asst. Prof. Botany, Univ. of Hawaii, Honolulu, T. H. Plant anatomy; plant physiology.

Evans, Clytee R., Prof. Biology, M.S.C.W., College Station, Columbus, Miss. Seed germination; plant ecology.

Eversole, H. O., Retired, 1856 Foothill Blvd., La Canada, Calif.

Eyster, H. Clyde, Asst. Prof. Botany, Univ. of South Dakota, Vermillion, S. Dak. Germination of seeds; osmotic relations.

Eyster, William H., Dept. of Botany, Bucknell Univ., Lewisburg, Pa. Genetics; plant pigments.

Ezell, Boyce D., Asst. Horticulturist, Bureau of Plant Industry Station, Beltsville, Md. Horticulture; fruit storage.

Fergus, E. N., Prof. of Farm Crops, Agronomist in Charge of Pasture and Forage Crop Investigations, Agri. Expt. Sta., Univ. of Ky., Lexington, Ky. Grass and clover improvement; pasture ecology.

Fife, J. M., Biochemist, Office of Sugar Plants, U.S.D.A., Box 31, Riverside, Calif. Plant biochemistry.

Flint, Lewis H., Professor of Botany, Louisiana State University, Baton Rouge, Louisiana.

Floyd, Bayard F., Horticulturist, Davenport, Fla. Citrus and other subtropical fruits in Florida; plant nutrition.

Fossum, M. Truman, Dept. of Horticulture, Univ. of Maryland, College Park, Md. Floriculture and horticulture.

Foster, A. Alfred, 2039 Richmond Ave., New Springville, Staten Island, New York.

Francis, W. D., Botanic Gardens, Brisbane, Australia. Protoplasm; iron utilization; chemosynthesis.

Frazier, John C., Asst. Prof. of Botany, Kansas State College and Asst. Plant Physiologist, Kansas Agr. Exp. Station, Manhattan, Kans. Plant chemistry; weed control.

Freeland, R. O., Assoc. Prof. Botany, Northwestern Univ., Evanston, Ill. Plant physiology; ecology.

French, C. S., Asst. Prof. of Botany, Dept. of Botany, University of Minnesota, Minneapolis, Minn. Photosynthesis.

Frenkel, Albert W., Kerckhoff Laboratories, California Inst. of Techonology, Pasadena, Calif. Photosynthesis.

Frey-Wyssling, Prof. Dr. Alb., Professor of General Botany and Plant Physiology, Swiss Federal Institute of Techonology, Zurich. Secretion and excretion; ultramicroscopic structure of protoplasm.

Furr, J. R., Physiologist, Bur. of Plant Industry, U. S. Subtropical Fruit Field Station, Orlando, Fla. Water relations.

Gail, Floyd W., Prof. Botany, Univ. of Idaho, Moscow, Idaho. Physiology; ecology.

Galligar, Gladys C., Asst. Prof. Biology, James Millikin Univ., Decatur, Ill. Tissue culture.

Garner, W. W., Physiologist, Bur. Plant Industry, Washington, D. C. Photoperiod; tobacco.

Gast, P. R., Asst. Prof. Forestry, Harvard Univ., Harvard Forest, Petersham, Mass. Forest tree growth and nutrition; radiation.

Gauch, Hugh G., Agent U. S. Regional Salinity Lab., Riverside, Calif. Plant nutrition; salt tolerance of plants.

Gerhardt, Fisk, Physiologist, U.S.D.A., Fruit Disease Lab., Box 99, Wenatchee, Wash. Physiology and biochemistry of deciduous fruits; transportation and storage.

Gerry, Eloise, Senior Microscopist in Forest Products, and Lecturer, Univ. of Wis., Forest Products Lab., Madison, Wis. Physiological plant anatomy; oleoresins and other secretions; heartwood formation.

Gibbs, R. Darnley, Asst. Prof. Botany, McGill Univ., Montreal, Quebec, Can. Tree physiology.

Gilbert, Basil E., Plant Physiologist, Director of Research, R. I. Agri. Expt. Sta., Kingston, R. I.

Gilbert, Seymour G., U. S. Field Laboratory for Tung Investigations. Gainesville, Fla.

Gilman, Joseph C., Prof. Botany, Iowa State College, Ames, Iowa. Physiology of fungi.

Girton, Raymond E., Prof. Plant Physiology, Dept. of Biology, Purdue Univ., Lafayette, Ind. Respiration of roots.

Glasstone, Violet F. C., Univ. of Oklahoma Research Inst., Norman, Okla.

Glatfelter, Edith E., Professor, Harris Teachers College, 4720 N. 20th St., St. Louis, Mo.

Glenister, Paul R., 6432 S. California Ave., Chicago, Ill. Mineral nutrition; respiration.

Goldsmith, G. W., Prof. Botany, Univ. of Texas, 14 Biology Bldg., Austin, Texas. Physiology of fungi.

Goodwin, R. H., Asst. Prof. Botany, Univ. of Rochester, Rochester, N. Y. Growth.

Gourley, Joseph H., Head Dept. of Horticulture, Ohio Agri. Expt. Sta., Wooster, Ohio. Horticulture.

Graber, L. F., Prof. Agronomy, Univ. of Wis., Madison, Wis. Food reserves; water utilization; cold resistance.

Granick, Sam, Asst. in Physical Chemistry, Rockefeller Inst. for Medical Res., 66th and York Ave., New York, N. Y. Oxidation reduction; enzymes.

Greathouse, Glenn A., Physiologist, Bur. of Plant Ind., U.S.D.A., Washington, D. C. Cold hardiness; chemistry of disease resistance; chemical and physical studies of cotton fiber.

Greene, Laurenz, Chief in Hort., Agri. Expt. Sta., Lafayette, Ind. Horticulture; floriculture; light.

Groner, Miriam G., 26 N. 4th St., Lewisburg, Pa.

Grossenbacher, Karl A., Biological Laboratories, Harvard University, Cambridge, Mass.

Gustafson, Felix G., Assoc. Prof. Botany, Univ. of Michigan, Ann Arbor, Mich. Parthenocarpy; growth; hormones; respiration.

Haas, A. R. C., Assoc. Plant Physiologist, Citrus Expt. Sta., Univ. of Calif., Riverside, Calif. Nutrition of citrus, avocado, walnut and date trees.

Hageman, Richard H., C. W. School, Edgewood, Md. Mineral nutrition.

Hahn, Vernon, Dept. of Conservation and Development, Trenton, N. J. Forest tree physiology.

Hamm, Philip C., Apt. 16, 1756 Grand, St. Paul, Minn. Mineral nutrition; plant propagation.

Hamner, Karl C., Plant Physiologist, U. S. Nutrition Laboratory, Ithaca, N. Y. Photoperiodism; plant hormones; plant nutrition.

*Hardy, Thora M. Plitt, Assoc. Microanalyst, Div. of Wildlife Research, Fish and Wildlife Serv., U. S. Dept. of the Interior, Beltsville, Md. Plant microchemistry, microscopy of furs.

Harley, C. P., Physiologist, U.S.D.A., in charge of Field Laboratory, Wenatchee, Wash. Fruit production and disease investigations.

Harris, G. Howell, Assoc. Prof. of Hort., Univ. of British Columbia, Vancouver, Canada. Plant nutrition; respiration and growth; food values of fruits and vegetables.

Harrison, Bertram F., Chairman, Botany Dept., Brigham Young Univ., Provo, Utah. Rooting of cuttings.

Harrison, C. M., Res. Assoc., Michigan State College, Farm Crops Dept., East Lansing, Mich. Pasture crops.

Hart, Helen, Asst. Prof. Plant Path., Univ. of Minn., University Farm, St. Paul, Minn. Disease resistance.

Hartman, John D., Res. Assoc. in Hort., Purdue Univ., Lafayette, Ind. Production, storage, processing and marketing of vegetable crops.

Hartman, H. T., Division of Pomology, Univ. of Calif., Davis, Calif.

Hartt, Constance E., Expt. Sta., Hawaiian Sugar Planters' Association, Honolulu, T. H. Sugar cane; nutrition; growth.

Harvey, E. M., Physiologist, U.S.D.A., 305 W. Alvarado, Pomona, Calif. Physiology and chemistry of fruiting.

Harvey, R. B., Prof. Plant Physiology, University Farm, St. Paul, Minn. Hardiness; light; mineral nutrition; storage.

Harvey, William A., Dept. of Agronomy, Washington State College, Pullman, Wash. Weed control.

Hassid, W. Z., Div. of Plant Nutrition, Univ. of Calif., Berkeley, Calif.

Hayward, Herman E., Senior Plant Anatomist, U. S. Regional Salinity Lab., Riverside, Calif. Developmental anatomy; nutrition; plant responses to saline conditions.

Heinicke, Arthur J., Prof. Pomology, N. Y. State College of Agri., Cornell Univ., Ithaca, N. Y. Plant physiology relating to fruit culture; photosynthesis.

Heinze, Peter H., U. S. Regional Vegetable Breeding Laboratory, Charleston, S. C. Photoperiodism.

Helgeson, E. A., Head Dept. of Botany, N. Dak. Agri. College, Fargo, N. Dak. Physiological ecology crop plants; seed germination.

Hendrickson, A. H., Pomologist, Univ. of Calif., Davis, Calif. Plant-soil-water relations.

Herrick, Ervin M., Commercial florist-grower, Hudson, Ohio. Cryoscopic methods; microchemistry; seed germination.

Hibbard, R. P., Plant Physiologist, Michigan State College, East Lansing, Mich.

Hiesey, W. M., Staff Member, Carnegie Inst., Stanford Univ., Calif. Comparative physiology of closely related climatic races.

Higinbotham, Noe, Texas Agricultural Exp. Station, Beaumont, Texas.

Hildreth, A. C., Guayule Research Project, P. O. Box 1708, Salinas, Calif.

Hill, George R., American Smelting and Refining Co., Salt Lake City, Utah. Photosynthesis.

Hill, Henry H., Va. Agri. Expt. Sta., Blacksburg, Va. Green manures nitrification; lysimeter experiments; soils.

Hires, Clara S., Owner, Mistaire Laboratories, 152 Glen Ave., Millburn, N. J. Raising plants in jellied chemicals.

Hoagland, D. R., Prof. Plant Nutrition, Chemist in Calif. Agri. Expt. Sta., 3048 Life Sciences Bldg., Berkeley, Calif. Inorganic nutrition; soil and plant relations.

Hobbs, Clinton H., 3023 Northwestern Ave., Indianapolis, Ind. Mineral nutrition; respiration.

Hock, Charles W., Textile Foundation, National Bur. of Standards, Washington, D. C. Protoplasm; cell wall.

Hoffer, George N., Mgr. Midwest Office, American Potash Inst., Inc., 423 Life Bldg., Lafayette, Ind. Plant nutrition.

Hope, Claude, Asst. Hort., U. S. Plant Introduction Garden, Glenn Dale, Md. Phytochemistry.

Horr, W. H., Assoc. Prof. Botany, Univ. of Kansas, Lawrence, Kans. Nutrition.

*Hottes, Charles F., Emeritus Prof. Plant Physiology, Dept. of Botany, Univ. of Ill., Urbana, Ill.

Howlett, Freeman S., Assoc. Hort., Ohio Agri. Expt. Sta., Wooster, Ohio. Physiology of reproduction in plants; development of gametes.

Hoxmeier, Sister Mary Claire, Biology Dept., Briar Cliff College, Sioux City, Iowa. Plant physiology.

Huber, Glenn A., Western Wash. Expt. Sta., Puyallup, Wash.

Huffington, Jesse M., Asst. Prof. Vegetable Gard. Extn., Hort. Bldg., State College, Pa. Vegetable growing; soil management.

Hurd-Karrer, Mrs. Annie May, Assoc. Physiologist, Div. of Cereal Inv., Bur. of Plant Ind., U.S.D.A., Washington, D. C. Toxic elements; plant nutrition.

Hutchins, Lee M., Div. of Forest Pathology, Bureau of Plant Industry Station, Beltsville, Md. Virus diseases; pathological diseases; soil oxygen.

Inge, Frederick D., Prof. Botany, Florida A. and M. College, Tallahassee, Fla. Plant growth and differentiation.

Ireland, J. C., Plant Physiologist, Okla. Agri. Expt. Sta., Stillwater, Okla. Plant breeding.

Issatchenko, B. L., Academy of Sciences, Ul. Kropotkina 16, Moskva, U.S.S.R. Microbiology; seeds.

Jacobson, H. G. M., Assoc. Soils Research, Conn. Agri. Expt. Sta., New Haven, Conn. Plant nutrition.

Jacobson, Louis, Analyst, Div. Plant Nutrition, Univ. of Calif., Berkeley, Calif. Physiology; nurition.

Jaffe, L. S., Jr., Microanalyst, Bur. Chemistry and Engr., U.S.D.A., Washington, D. C. Absorption phenomena.

Jayne, Martha, Army Medical School, Washington, D. C.; R.F.D. 1, Laceyville, Pa. Growth and movement; metabolism.

Jeffrey, R. N., Plant Physiologist, Ky. Agri. Expt. Sta., Lexington, Ky. Physiology of tobacco curing; photosynthesis; enzymes; pectin.

Jennings, O. E., Prof. Botany, Univ. of Pittsburgh, Pittsburgh, Pa.

Joham, Howard E., Junior Physiologist, 703A E. 31st, Bryan, Texas. Nutritional studies.

Johnson, Edna L., Prof. Biology, Univ. of Colo., Boulder, Colo. Radiation of plants; growth-promoting substances.

Johnston, Earl S., Asst. Director, Div. of Radiations and Organisms, Smithsonian Inst., Washington, D. C. Radiation and plant growth.

Johnstone, G. R., Univ. of South. Calif., 3551 University Ave., Los Angeles, Calif. Reproduction of marine algae.

Jones, John P., Soil Conservation Service, Center Building, Upper Darby, Pa.

Jones, Linus H., Asst. Res. Prof. Botany, Mass. State College, Amherst, Mass. Plant nutrition; soil temperature effects on plant growth.

Jones, Winston W., Associate Horticulturist, University of Ariz., Tucson, Ariz. Respiration; fruit storage.

Jurica, Hilary S., St. Procopius College, Lisle, Ill. Permeability.

Keenan, Edward T., Keenan Soil Lab., Frostproof, Fla. Plant nutrition.

Kersten, Harold J., Assoc. Prof. Biophysics and Fellow in the Graduate School, Univ. of Cincinnati, Cincinnati, Ohio. Biological effects of radiation.

Kertesz, Z. I., Chief in Res. Chem., N. Y. State Agri. Expt. Sta., Geneva, N. Y. Plant enzymes; pectins; plant biochemistry.

Kezer, Alvin, Prof. of Agronomy, Colo. Agri. College, Fort Collins, Colo. Nitrogen metabolism.

Kimbrough, W. D., Dept. of Hort., Univ. of La., University, La. Horticulture.

King, Genevieve, Department of Botany, Marshall College, Huntington, W. Va. Floral primordia; seedless fruits; morphogenic responses.

Kitchen, E. M., Pacific Coast Borax Co., 51 Madison Ave., New York, N. Y. Boron.

Knott, James E., Head Div. of Truck Crops, Univ. of Calif., Davis, Calif. Photoperiodism and temperature relationships.

Korstian, Clarence F., Director School of Forestry, Duke Univ., Durham, N. C. Silvics.

Kozlowski, T. T., School of Forestry, Duke University, Durham, N. C.

Kramer, Amihud, Res. Asst., Nat'l Canners Assoc., 1739 H St. N.W., Washington, D. C. Physiological changes associated with maturation.

Kramer, Paul J., Assoc. Prof. Botany, Duke Univ., Durham, N. C. Absorption of water; physiology of woody plants.

Kraus, E. J., Prof. and Chair. Dept. of Botany, Univ. of Chicago, Chicago, Ill. Morphology; histology; nutrition.

Kraybill, H. R., Dept. of Scientific Research, American Meat Institute, 59 East Van Buren St., Chicago, Ill.

Krotkov, G., Asst. Prof. Biology, Queen's Univ., Kingston, Ontario, Can. Respiration; cold and gas storage.

LaFuze, H. H., Assoc. Prof. Biology, E. Ky. State College, Richmond, Ky. Nutrition; physiology of fungi.

Latimer, Lorenzo P., Dept. of Hort., Univ. of New Hamp., Durham, N. H. Pollination; electrical conductivity in relation to growth and keeping qualities of fruits.

Lauritzen, John I., Bureau of Plant Industry Station, Beltsville, Md. Storage.

Leach, William, Head Dept. Botany, Univ. of Manitoba, Winnipeg, Manitoba. General physiology; plant respiration.

LeClerg, E. L., Pathologist, U.S.D.A., Hort. Research, University, La. Plant pathology.

LeCompte, Stuart B., Jr., Asst. Plant Physiologist, Conn. Agri. Expt. Sta., Windsor, Conn. Soil conditions; plant pigments.

Leng, Herta, Dana Hall School, Wellesley, Mass. Metablism: radioactive elements as tracers.

Leonard, Oliver A., Asst. Plant Physiologist, Miss. Agri. Engr. Expt. Sta., State college, Miss. Root ecology; weed control; translocation.

Leonian, Leon H., Prof. Mycology, W. Va. Univ., Morgantown, W. Va. Physiology of fungi.

Levitt, J., Department of Botany, University of Missouri, Columbia, Mo. Frost resistance; cell permeability.

Lewis, James C., Biochemical Division, Western Regional Research Lab., Albany, Calif.

Lindner, Robert C., U. S. Fruit Disease Laboratory, Box 99, Wenatchee, Wash. Mineral nutrition; growth.

Linford, M. B., Research Institute, P. O. Box 3166, Honolulu, T. H.

Lipman, Charles B., Prof. Plant Physiology and Dean of the Graduate School, Univ. Calif., Berkeley, Calif. Mineral metabolism of higher plants; nitrogen fixation; bacterial physiology.

‡Livingston, Burton E., Prof. Emer. Johns Hopkins Univ., Riderwood, Md., Physiology and its applications, especially physical aspects; soil and climatic conditions as related to plants.

*Lloyd, Francis E., Prof. Emer. McGill Univ., Box 482, Carmel, Calif. Stomata; rubber.

Lodder, William B., Chemist, P. O. Box 292, Syracuse, N. Y. Plant nutrition and fertilizer chemistry.

‡Loehwing, Walter F., Prof. and Head, Dept. of Botany, Univ. of Iowa, Iowa City, Iowa. Plant development and nutrition.

Long, David D., Director International Minerals and Chemical Corporation, 20 N. Wacker Drive Bldg., Chicago, Ill.

Long, Frances L., Ecological Investigator, Carnegie Inst. of Washington, 3111 Argonne Circle, Santa Barbara, Calif. Photosynthesis; stomata; pollination.

Longnecker, Mayne, Assoc. Prof. Biology, Southern Methodist Univ., Dallas, Texas. General physiology.

Loomis, Walter E., Assoc. Prof. Plant Physiology, Iowa State College, Ames, Iowa. Absorption of energy by leaves; physiology of maize.

Lorenz, Ralph W., Assoc. in Forest Research, Univ. of Ill., Urbana, Ill. Silvics; high temperature tolerance in forest trees.

Lund, E. J., Prof. Physiology, Univ. of Texas, Austin, Texas. Bioelectric potential; growth.

Lutman, B. F., Vermont Agri. Expt. Sta., Burlington, Vt.

Lutz, J. M., Associate Physiologist, U. S. Hort. Field Station, Meridian, Miss. Transportation and storage of fruits and vegetables.

Luyet, B. J., Prof .of Botany, School of Medicine, St. Louis Univ., St. Louis, Mo. Latent life at low temperature and in the dry state; biophysics.

Lyon, Channing B., Associate Plant Physiologist, U. S. Nutrition Laboratory, Ithaca, N. Y.

Lyon, Charles J., Prof. Botany, Dartmouth College, Hanover, N. H. Growth rings of trees; osmotic relations; teaching methods in plant physiology.

McAllister, F., Prof. Botany, Univ. of Texas, Austin, Texas. Morphology of Bryophyta; starch deposition.

McCall, M. A., Principal Agronomist in Charge Division of Cereal Crops and Diseases; Asst. Chief, Bur. of Plant Ind., U.S.D.A., Washington, D. C. Physiology; anatomy.

McComb, A. L., Asst. Prof. Forestry, Iowa State College, Ames, Iowa. Nutrition and growth requirements of trees.

McDermott, J. Joseph, Detroit Rex Products Co., Detroit, Mich. Plant physiological chemistry.

*MacDougal, Daniel T., R.F.D. 170, Carmel, Calif. Growth; general.

McGeorge, W. T., Agricultural Chemist, Head of Dept., Ariz. Agri. Expt. Sta., Tucson, Ariz. Soils and plant nutrition.

MacGillivray, John H., Asst. Prof. Truck Crops and Assoc. Olericulturist, Davis, Calif. Physiology of truck crops.

McGinnies, W. G. Emergency Rubber Project, 315 W. 9th St., Los Angeles, Calif. Physiological ecology.

McIlvanie, Samuel K., Soil Conservation Service, Box 299, Ekalaka, Montana. Reactions to disturbance.

McKee, Mary C., Prof. Chemistry, Connecticut College, New London, Conn. Organic and biochemistry.

MacKinney, Gordon, Asst. Prof. Fruit Technology, Univ. of Calif., Berkeley, Calif. Plant pigments.

McKinney, H. H., Senior Pathologist, Bureau of Plant Industry Station, Beltsville, Md. Viruses; virus diseases; ecological physiology.

McLarty, H. R., Officer-in-charge, Dom. Lab. of Plant Path., Summerland, B. C., Can. Physiological disorders of plants.

McLean, R. C., Prof. of Botany, University College, Newport Rd., Cardiff, Wales.

MacMasters, Majel M., Assoc. Chemist Starch and Dextrose Div., North. Regional Res. Lab., Peoria, Ill. Starch granule structure; amylase action, etc.

McMurtrey, J. E., Jr., Senior Physiologist, Tobacco Inves., Bur. of Plant Ind. Station, Beltsville, Md. Mineral nutrition; biochemistry and biophysics.

Machlis, Leonard, Guayule Emergency Rubber Project, P. O. Box 1708, Salinas, Calif.

Macht, David I., Dir. Pharmacological Res. Lab., Hynson, Westcott and Dunning, Charles and Chase Sts., Baltimore, Md. Phytopharmacology or effects of drugs on plants.

Mack, Warren B., Head Dept. of Hort., Penn. State College, State College, Pa. Nutrition of vegetable crops.

Mackie, William H., Univ. of Calif., 124 Hilgard Hall, Berkeley, Calif.

Macy, Paul, Associate Chemist, Picatinny Arsenal, Dover, N. J.

Mader, E. O., R.F.D. 1, Doylestown, Ohio.

Madson, B. A., Prof. Agronomy, University Farm, Davis, Calif. Carbohydrate and protein nutrition of plants.

Magistad, O. C., U. S. Regional Salinity Lab., Box 672, Riverside, Calif. Tropical soils; soil salinity and relation to plant growth.

Magness, J. R., Princ. Pomologist, Bureau of Plant Industry Station, Beltsville, Md. Physiology of growth and storage.

Mahoney, Charles H., Dept. of Hort., Univ. of Maryland, College Park, Md. Storage of celery and onions.

Maki, T. Ewald, Senior Forester, Beltsville Forest Laboratory, R.F.D. 2, Laurel, Md. Tree physiology; forest soils and tree growth.

Malan, F. P., Stellenbosch, Cape Province, South Africa.

Mandels, Gabriel R., 41 Stewart Ave., Waterbury, Conn.

Marsh, Gordon, Asst. Prof. of Zoology, State Univ. of Iowa, Iowa City, Iowa. Bioelectricity and metabolism.

Marshall, J. B., Jr. Res. Biologist, Div. of Biology and Agri., Nat'l Res. Council, Ottawa, Can. Growth studies under controlled environmental conditions.

Martin, Emmett, Carnegie Inst. of Washington, Stanford Univ., Palo Alto, Calif.

Marvin, James W., Box 204A, R.F.D. 1, Burlington, Vt. Cell shapes, morphogenesis.

Mason, Thomas Godfrey, Head Physiologist, Empire Cotton Growing Corp., Trinidad, B.W.I. Hydration.

Massey, L. M., Prof. Plant Path. and Head of Dept., N. Y. State College of Agri., Cornell University, Ithaca, N. Y. Pathology.

†Maximov, N. A., Academy of Sciences Ul. Kropotkina 16, Moskva, U.S.S.R. Water relations; drought and frost resistance; plant development.

Mehrlich, Ferdinand P., Hawaiian Pineapple Co., Ltd., P. O. Box 3380, Honolulu, T. H.

Meier, Henry F. A., Head, Dept., Forestry, Botany and Pathology, N. Y. State College of Forestry, Syracuse, N. Y. Mineral requirements; protoplasm; growth promoting substances.

Meredith, Clifford H., Plant Pathologist, Science Master of Friends College, Highgate, Jamaica, B. W. I. The effect of various chemicals on the growth of banana roots in the soil.

Mes, Margaretha Gerarda, Sr. Lecturer in Botany, Univ. of Pretoria, Pretoria, S. Africa. Photosynthesis; transpiration and water-relations; influence of toxins secreted by fungi on growth.

Meyer, Bernard S., Prof. Botany, Ohio State Univ., Columbus, Ohio. Water relations; photosynthesis.

Meyer, Samuel L., Asst. Prof. Botany, Univ. of Tenn., Knoxville, Tenn. Bryophyte physiology.

Miller, Edwin C., Prof. Plant Physiology, Botany Dept., Kansas State Agri. College, Manhattan, Kans. Water relations; plant chemistry.

Miller, Erston V., Physiologist, Bur. of Plant Ind., U.S.D.A., 415 N. Parramore St., Orlando, Fla. Biochemistry of fruits and vegetables in storage and transportation; plant pigments.

Miller, Laurence P., Biochemist, Boyce Thompson Inst. for Plant Res., Yonkers, N. Y. Effect of chemical stimulants on plant metabolism; glucoside formation in plants.

Mitchell, H. L., Lake City, Fla. Nutrition; soils, silvics; tree physiology; forest influences.

Moinat, Arthur D., Asst. Prof. Biology, Colo. State College Branch, Hesperus, Colo. Water relations; growth.

Moir, W. W. G., Agri. Technologist, Box 2298, Honolulu, T. H. Nutrition.

Montermoso, Juan C., Asst. Rubber Technologist, Rubber Laboratory, U. S. Navy Yard, Mare Island, Calif., 2418 Bancroft Way, Berkeley, Calif. Transpiration.

Moore, Dwight M., Prof. Botany, Univ. of Ark., Fayetteville, Ark. Rest period; flora of Arkansas.

Moreland, C. F., Dept. of Botany, Univ. of La., University, La.

Morris, H. E., Head, Dept. Botany and Bacteriology, Montana State College, Bozeman, Mont. Mineral deficiencies in relation to plant diseases.

Muenscher, W. C., Prof. Botany, Cornell Univ., Ithaca, N. Y. Germination and dormancy of seeds; weed control methods.

Mullison, Wendell R., Dept. of Biology, Purdue Univ., Lafayette, Ind.

Murneek, A. E., Assoc. Prof. Horticulture, Univ. of Missouri, Columbia, Mo. Reproduction; photoperiodism; correlation.

Murphy, (Rev.) John B., Prof. Botany, DePaul Univ., 1010 Webster Ave., Chicago, Ill. General botany; permeability.

Musser, A. M., Horticulturist, S. C. Agri. Expt. Sta., Clemson College, S. C. Peach stocks.

Myers, Jack, Dept. of Zoology, Univ. of Texas, Austin, Texas. Photosynthesis; plant pigments; physiology of chlorella.

Naylor, A. W., Instructor Botany, Univ. of Chicago, Chicago, Ill. Photoperiodism; nutrition; growth.

Nelson, Clarence H., 32 E. Bloomington St., Iowa City, Iowa.

Nelson, H. C., 8329 Wabash Ave., Grand Crossing Sta., Chicago, Ill.

Nelson, R. C., Research Laboratory, Armour and Co., Chicago, Ill. Ripening of fruits; boron nutrition.

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Noecker, N. L., Instructor Biology, Univ. of Notre Dame, Notre Dame, Ind. Vitamins; physiology of fungi.

Noggle, G. Ray, Fellow in Botany, Univ. of Illinois, Urbana, Ill. Plant physiology; plant vitamins and hormones; nitrogen metabolism.

Norman, A. G., Prof. Agronomy, Iowa State College, Ames, Iowa. Structural constituents of plants; plant analysis.

Northen, Henry T., Asst. Prof. Botany, Univ. of Wyoming, Laramie, Wyo. Protoplasm structure; irritability.

Olmsted, Charles E., Dept. of Botany, Univ. of Chicago, Chicago, Ill. Ecology of range grasses; interactions between plants in deciduous forests; water relations.

Oserkowsky, Jacob, Univ. of Calif., 110 Hilgard Hall, Berkeley, Calif. Growth; chlorosis.

*Osterhout, W. J. V., Rockefeller Medical Foundation, 66th St. and York Ave., New York, N. Y. Permeability.

Ostrom, Carl E., Beltsville Research Center Experimental Forest, R.F.D. 2, Laurel, Md. Forest tree physiology.

Overcash, J. P., West Tennessee Expt. Station, Jackson, Tenn.

Overley, Fred L., Agri. Expt. Sta., Wenatchee, Wash.

Owen, F. V., Geneticist, U.S.D.A., 1810 S. Main St., Salt Lake City, Utah.

Owens, J. S., Prof. Agronomy, Univ. of Conn., Storrs, Conn. Tobacco curing; preservation of forage crops.

Paddick, Morris E., Asst. Prof. Plant Physiology, Dept. of Botany, Colo. State College, Fort Collins, Colo. Growth and nutrition.

Palmer, E. F., Hort. Expt. Sta., Vineland Sta., Ontario, Can.

Parker, M. W., Physiologist, Bureau of Plant Industry Station, Beltsville, Md. Photoperiodism; biochemistry.

Patterson, C. F., Dept. of Horticulture, Univ. of Saskatchewan, Saskatoon, Sask, Can.

Pearse, H. L., Chief Plant Physiologist, Western Prov. Fruit Res. Sta., Stellenbosch, C. P., S. Africa. Nutrition and physiology of fruit trees; respiration; plant hormones.

*Peirce, George J., Prof. Emer. Botany and Plant Physiology Stanford Univ., 281 Embarcadero Rd., Palo Alto, Calif. Smoke and cement dust injury; ascent of sap.

Pentzer, W. T., Plant Physiologist, U.S.D.A., Rt. 3, Box 552, Fresno, Cal. Respiration of fruits and vegetables; carbohydrates; metabolism.

Pepkowitz, Leonard, Dept. of Agric. Chemistry, Agr. Experiment Station, Kingston, R. I. Nutrition and metabolism.

Pessin, L. J., Research in Silvics, Federal Bldg., New Orleans, La. Tree nutrition; growth and reproduction.

Peters, Charles A., Prof. Inorganic and Soil Chemistry, Mass. State College, Amherst, Mass. Iron in nutrient solutions.

Phillips, Thomas G., Prof. Agri. and Biol. Chemistry and Chemist in the Expt. Sta., Univ. of N. H., Durham, N. H. Physiological chemistry.

Pierre, W. H., Agronomist and Head of Agronomy Dept., Iowa State College, Ames, Iowa. Soil plant relationships; plant nutrition.

Platenius, Hans, Asst. Prof. Vegetable Crops, Cornell University, Ithaca, N. Y. Plant chemistry; storage.

Popp, H. W., Prof. Botany, Pennsylvania State College, State College, Pa. Radiation.

Post, Francis A., Soil Conservation Service, R.F.D. 3, Zanesville, Ohio. Water relations of plants.

Pratt, Dudley J., 1118 Taylor St., Topeka, Kansas. Plant physiology.

Pratt, Harlan K., 146 Physics-Biology Building, Univ. of Calif., Los Angeles, Calif.

Pratt, Robertson, Instructor, Univ. of Calif. Medical Center, San Francisco, Calif. Growth; medicinal plants; auxins.

Prianischnikov, D. N., Academy of Sciences, Ul. Kropotkina 16, Moskva, U.S.S.R.

Pucher, Geo. W., Res. Assoc. in Biochemistry, Conn. Agri., Expt. Sta., New Haven, Conn. Organic acid and nitrogen metabolism.

Pulling, Howard E., Prof. Botany, Wellesley College, Wellesley, Mass. Plant physiology.

Pultz, L. M., Prof. and Head of Botany and Range Ecology, Univ. of Ariz., Tucson, Ariz. Carbohydrates.

Quillian, Marvin C., Prof. Biology, Wesleyan College, Macon, Ga. Morphology; physiology; ecology.

Raines, M. A., Prof. Botany, Howard Univ., Washington, D. C. Root systems; techniques and instrumentation.

Raleigh, George J., Dept. of Vegetable Crops, Cornell Univ., Ithaca, N. Y. Seeds; plant nutrients.

Raleigh, S. M., Assoc. Prof., Univ. of Maine, Orono, Maine. Plant nutrition.

Reed, H. S., Professor of Plant Physiology, Division of Plant Nutrition, Univ. of Calif., Berkeley, Calif.

Reeve, Eldrow, Dept. of Botany, Rutgers University, New Brunswick, N. J.

Reid, Mary E., National Institute of Health, Bethesda, Md. Mineral nutrition; vitamins, plant growth factors.

Reuther, Walter, Horticulturist, U. S. Date Garden, Indio, Calif. Photosynthesis; mineral nutrition; water relations.

Richards, F. J., Rothamsted Expt. Sta., Harpenden, Herts, England. Mineral nutrition.

Richey, Frederick D., Agricultural Experiment Station, Knoxville, Tenn. Breeding and growth of maize.

Rigg, Geo. B., Prof. Botany and member of staff in Oceanography, Univ. of Wash., Seattle, Wash. Physiology of marine plants; physiology of plants of sphagnum bogs.

Robbins, W., Rei, Asst. Plant Physiologist, N. J. Agri. Expt. Sta., New Brunswick, N. J. Mineral nutrition; nitrogen metabolism.

Roberts, Edith A., Chairman, Dept. of Botany, Vassar College, Poughkeepsie, N. Y. Physiology.

Roberts, John W., Principal Pathologist, Bureau of Plant Industry Station, Beltsville, Md. Diseases of deciduous fruit trees.

Roberts, R. H., Prof. Horticulture, Univ. of Wis., Madison, Wis. Physiology; anatomy.

Roddis, Lovis H., Bur. of Medicine and Surgery, Navy Dept., Washington, D. C.

Rohrbaugh, Lawrence M., Dept. of Botany, Univ. of Oklahoma, Norman, Okla. Plant nutrition; effects of light quality.

Rohrbaugh, P. W., Plant Physiologist, Res. Dept., Calif. Fruit Growers Exchange, 616 N. Grove St., Ontario, Calif. Physiology of citrus fruit; respiration.

Rosene, Hilda F., Asst. Prof. Physiology, Univ. of Texas, Austin, Texas. Bioelectric potentials; water relations; growth.

Runyon, Ernest H., Assoc. Prof. Botany, Agnes Scott College, Decatur, Ga. Water relations; organization in Dictyostelium.

†Russell, Sir E. John, Dir. Rothamsted Expt. Sta., Harpenden, England. Soil conditions and plant development.

Rygg, G. L., 733 W. Orange Grove Ave., Pomona, Calif.

Sachs, Ward H., Belmont Apt. 6, Adams and Park Place, Wilmington, Del.

Sackston, Waldemar E., Dominion Laboratory of Plant Pathology, Winnipeg, Can. Water relations; physiology of parasitism.

Saeger, Albert C., Chairman, Dept. of Biology, Jr. College of Kansas City, Kansas City, Mo.

Salmon, S. C., Div. of Cereal Crops, U.S.D.A., Washington, D. C. Agronomy.

Sampson, A. W., 305 Hilgard Hall, Univ. of Calif., Berkeley, Calif.

Sampson, Homer C., Prof. Botany, Ohio State Univ., Columbus, Ohio. Physiology; ecology.

Sayre, Jasper D., Physiologist, Div. of Cereal Crops and Diseases, Ohio Agri. Expt. Sta., Wooster, Ohio. Stomata; physiology of corn.

Scarth, Geo. W., Macdonald Prof. of Botany, McGill Univ., Montreal, Can. Physiology and its application.

Schlenker, Frank S., Asst. Chemist, R. I. Agri. Expt. Sta., Kingston, R. I. Solution culture and nitrogen metabolism.

Schopfer, William Henry, Institut Botanique, Berne, Switzerland. Sex of fungi; nutrition; vitamins.

Schopmeyer, Clifford S., Olustee Experimental Forest, Lake City, Fla. Water relations; reforestation; vegetative propagation of pines.

Schrader, Albert Lee, Prof. Pomology, Dept. of Hort., Univ. of Maryland, College Park, Md. Cultural requirements and physiology of fruits.

Schultz, Orville C., Assoc. Prof. Botany, Okla. A. & M. College. Stillwater, Okla. Physiology.

Schuster, C. E., Hort. Div., Oregon State College, Corvallis, Ore.

Scofield, Herbert T., Asst. Prof. Botany, Ohio State Univ., Columbus, Ohio. Light relations; organic nutrition; ecology.

Seifriz, William, Prof. Botany, Univ. of Penna., Philadelphia, Pa. Protoplasm.

Severy, J. W., Chairman, Division of Biology, State Univ., Missoula, Mont. Plant physiology; ecology of flora of Montana.

*Shantz, H. L., Chief, Div. of Wildlife Management, Forest Service, U.S.D.A., Washington, D. C. Plant geography; wildlife management.

Shaw, Seth T., Dept. of Hort., Brigham Young Univ., Provo, Utah.

Shear, G. M., Assoc. Plant Physiologist, Va. Agri. Expt. Sta., Blacksburg, Va. Nutritional diseases; effects of pH on growth.

Shirley, Hardy L., Dir. Allegheny Forest Expt. Sta., 622 Bankers Securities Bldg., Philadelphia, Pa. Tree physiology; drought and cold resistance; light and growth.

Shive, John W., Plant Physiologist, N. J. Agri. Expt. Sta., and Prof. Plant Physiology, Rutgers Univ., New Brunswick, N. J. Plant nutrition.

‡Shull, Charles A., Prof. Plant Physiology, Univ. of Chicago, Chicago, Ill. Water relations; mineral nutrition; radiation; growth phenomena.

Shull, George H., Prof. Botany and Genetics; 60 Jefferson Rd., Princeton, N. J. Genography; species hybridization; physiological actions and reactions of genes.

Sideris, Christos P., Univ. of Hawaii, Honolulu, T. H.

Siems, H. B., Chief Chemist, Fertilizer Dept., Swift and Co., Chicago, Ill. General.

Sinclair, Walton B., Asst. Prof. Plant Physiology, Univ. of Calif. Citrus Expt. Sta., Riverside, Calif. Plant biochemistry.

Singh, Bhola N., Head of the Inst. of Agri. Research, Benares Hindu Univ., Benares, India. Plant physiology; agronomy; biochemistry.

Sitton, Benjamin G., Assoc. Pomologist, U.S.D.A., P. O. Box 812, Bogalusa, La. Pecan production investigations; propagation, especially grafting; horticulture.

Skirm, George W., Plant Breeder, L. Sherman Adams Co., Wellesley, Mass. Inorganic nutrition.

Skok, John, Grad. Asst. in Botany, Univ. of Chicago. Chicago, Ill. Plant nutrition.

Small, James, Prof. Botany, Queen's Univ., Belfast, Ireland, General; pharmacognosy.

Small, John A., Asst. Prof. Botany, N. J. College for Women, New Brunswick, N. J.

Smith, C. L., Physiologist, U.S.D.A., Brownwood, Texas. Physiology and biochemistry of the pecan.

Smith, James H. C., Staff Member, Carnegie Inst., Stanford Univ., P. O., Calif. Photosynthesis.

Smith, John B., Chemist and Assoc. Prof. Agri. Chemistry, R. I. State College, Agri. Expt. Sta., Kingston, R. I. Plant chemistry; soils, soil fertility.

Smith, Malcolm E., Div. of Plant Nutrition, Univ. of Calif., Berkeley, Calif. Mineral nutrition, especially the function of boron in the plant.

Sokoloff, V. P., Junior Chemist, Citrus Experiment Station, Riverside, Calif. Reaction of roots to various forms of nitrogen.

Somers, G. F., Dairy Bldg., New York State College of Agriculture, Ithaca, N. Y.

Sommer, Anna L., Assoc. Soil Chemist, Ala. Agri. Expt. Sta., Auburn, Ala. Inorganic nutrition of plants; photosynthesis.

Spencer, Ernest L., Dept. of Agronomy, Rutgers University, New Brunswick, N. J. Plant nutrition; physiological pathology.

Sperti, George Speri, Dir. of Scientific Res. and Educ., Institutum Divi Thomae, Mt. Washington, Cincinnati, Ohio. Cancer research.

Spessard, E. A., Hendrix, College, Conway, Ark.

‡Spoehr, Herman A., Carnegie Institution of Washington, Stanford Univ., Calif. Physiology.

*Sponsler, O. L., Prof. Botany, Univ. of California, Los Angeles, Calif. Structure of proteins.

Sprague, G. F., Agronomist, U.S.D.A., Iowa State College, Ames, Iowa. Genetics; corn breeding; physiology.

Sprague, Howard B., Agronomist, Prof. Agronomy, Rutgers Univ., New Brunswick, N. J. Ecology of field crops; chlorophyll inheritance; breeding crop plants; grassland culture.

Sprague, Vance G., Assoc. Agronomist, U. S. Regional Pasture Research Lab., State College, Pa. Physiology.

Stahl, Arthur L., Assoc. Hort., Agri. Expt. Sta., Univ. of Fla., Gainesville, Fla. Preservation of foods; plant nutrition; composition of foodstuff.

Stanfield, J. Fisher, Dept. of Science, Chicago Teachers College, Chicago, Ill. Physiology of sex; vitamins.

Stark, Arvil L., Ext. Hort., Utah State College, Logan, Utah.

Steinbauer, C. E., U.S.D.A., Rubber Field Laboratory, University Farm, St. Paul, Minn.

Steinbauer, George P., Assoc. Prof. Botany and Seed Analyst, Univ. of Maine, Orono, Me. Seed germination; physiology of dormancy.

Steinberg, Robert A., Assoc. Physiologist, U.S.D.A., Washington, D. C. Plant nutrition.

Steinmetz, F. H., Prof. Botany, Univ. of Maine, Orono, Maine. Winter hardiness.

Steward, F. C., Dept. of Botany, Birkbeck College, Univ. of London, London, England. General physiology; biochemistry.

Steyaert, René L., Labatoire de Bambesa, District de l'Vele, Congo Belge, Africa.

Stier, H. L., Asst. Olericulturist and Instructor, Dept. of Hort., Univ. of Maryland, College Park, Md. Physiology of vegetable crops; nutrition of vegetable crops; plant growth substances.

Stirling, James, Lecturer, Dept. of Botany, The University, Liverpool, England. Growth.

Stoldt, Elnore, 759 S. Church St., Jacksonville, Ill.

Stoller, B. B., L. F. Lambert Co., Coatesville, Pa. Physiology of the mushroom.

Stor, Paul W., Science Dept., Concordia College, St. Paul, Minn.

Stoutemeyer, V. T., Associate Horticulturist, U. S. Plant Introduction Garden. Glenn Dale, Md. Phytohormones; regeneration in plants.

Strausbaugh, P. D., Prof. Botany, W. Va. Univ., Morgantown, W. Va. Dormancy of seeds, buds; phytohormones; winter hardiness.

Street, Orman E., Plant Physiologist, Tobacco Expt. Sta., Lancaster, Pa.

Stuart, Neil W., Assoc. Physiologist, Bureau of Plant Industry Station, Beltsville, Md. Physiology of floricultural crops.

Stuckey, Irene, Asst. Plant Physiologist, R. I. Expt. Sta., Kingston, R. I. Low temperatures; pasture grasses.

Sullivan, J. T., Physiologist, U. S. Pasture Lab., State College, Pa. Biochemistry.

Swanson, Carrol A., Botany Dept., Ohio State Univ., Columbus, Ohio. Transpiration; photosynthesis.

Swingle, Charles F., Horticulturist, Soil Conservation Service, Manhattan, Kans. Erosion control plants; propagation; hardiness.

Sylvain, Pierre G., Pan-American Fellow, Dept. of Botany, Iowa State College, Ames, Iowa. Hormones, growth correlations and photoperiodism.

Teakle, L. J., Research Officer and Adviser in Plant Nutrition, Dept. of Agri., Perth, W. Australia. Plant nutrition; soil science.

Tengwall, T. A., Research Dept., Firestone Plantations Co., Monrovia, Liberia.

Thimann, Kenneth V., Assoc. Prof. Plant Physiology, Harvard Univ., Cambridge, Mass. Growth; growth hormones; vegetative propagation; plant biochemistry; metabolism of microorganisms.

Thoday, D., Dept. of Botany, University College of North Wales, Bangor, N. Wales, England.

Thomas, Meirion, Dept. of Botany, King's College, New Castle-on-Tyne 2, England. Chemical problems of plant physiology.

Thomas, Roy C., Assoc. Plant Pathologist, Ohio Agri. Expt. Sta., Wooster, Ohio. Plant lysins; bacterial diseases.

‡Thomas, Walter, Prof. Plant Nutrition, Pennsylvania State College, State College, Pa. Foliar diagnosis.

Thompson, Homer C., Prof. and Head of Dept. of Vegetable Crops, N. Y. State College of Agri, Ithaca, N. Y. Temperature and light; minor or micro elements.

Thompson, Ross C., Assoc. Hort., Bureau of Plant Industry Station, Beltsville, Md. Genetics and breeding of lettuce.

Thone, Frank, Biology Editor, Science Service, Washington, D. C. Water relations; atmometry; science popularization.

Thornton, S. F., Dir. Res. and Farm Serv. Dept., F. S. Royster Guano Co., Norfolk, Va. Fertilizers; plant nutrition; soil chemistry.

Thut, Hiram F., Asst. Prof. Botany, East. Ill. State Teachers College, Charleston, Ill. Transpiration; growth; teaching of botany.

Tiffany, (Lewis) Hanford, Prof. Botany and Chairman Dept. of Botany, Northwestern Univ., Evanston, Ill. Physiology and ecology of algae; Oedogoniales; teaching of botany.

Ting, Sik-Vung, Horticulture Dept., Ohio State University, Columbus, Ohio. Growth promoting substances.

Tottingham, W. E., Assoc. Prof. Biochemistry, Univ. of Wis., Madison, Wis. Polyphenolase system of potato tuber; the function of boron in plants.

Traub, Hamilton P., Guayule Emergency Rubber Project, Box 1708, Salinas, Calif. Phytohormone research.

Trelease, Sam F., Prof. Botany, Columbia Univ., New York, N. Y. Nutrition.

Tubbs, F. R., Plant Physiologist, Tea Res. Inst. of Ceylon, St. Coombs, Talawakelle, Ceylon. Physiology of crop plants.

Tufts, Warren P., Prof. Pomology, Univ. of Calif., University Farm, Davis, Cal. Climate in relation to fruit growing; fruit morphology; pruning.

Tukey, H. B., Chief in Research, N. Y. State Agri. Expt. Sta., Geneva, N. Y. Horticulture; developmental morphology; plant propagation.

Turrell, E. M., Jr. Plant Physiologist, Univ. of Calif. Citrus Expt. Sta., Riverside, Calif. Physiology of citrus.

Twining, Frederick E., Owner Twining Laboratories, Fresno, Calif. P. O. Box 147.

Ulrich, Albert, Div. of Nutrition, Univ. of Calif., Berkeley, Calif.

†Ursprung, Alfred, Director Botanical Inst., Fribourg, Switzerland.

Vandecaveye, S. C., Dept. of Agronomy, State College of Wash., Pullman, Wash.

Van Haarlem, J. R., Asst. in Res., Vineland Sta., Ontario, Can.

Van Horn, Clifton, W., Asst. Hort., Univ. Expt. Sta., Yuma, Ariz. Physiology of fruiting in horticultural trees.

Van Niel, C. B., Professor of Microbiology, Hopkins Marine Station, Pacific Grove, Calif. General Biochemistry.

Van Overbeek, J., Plant Physiologist, Institute of Tropical Agriculture, Mayaguez, Puerto Rico. Physiology of growth, growth hormones; physiology of aquatic plants.

Vaughan, Edward K., Assoc. Prof. of Biology, Virginia Polytechnic Inst., Blacksburg, Va. Physiology of plant pathogenic microorganisms.

Veerhoff, Otto (Jr.), Horticultural Physiologist, N. C. State College, Raleigh, N. C. Mineral nutrition; germination.

Veihmeyer, Frank J., Prof. of Irrigation, Univ. of Calif., Davis, Calif. Plant-soil-water relations.

Vickery, Hubert B., Biochemist in charge, Biochemical Laboratory, Conn. Agri. Expt. Sta., New Haven, Conn. Proteins; amino acids; nitrogen metabolism.

Vlamis, James, Assistant, The Harvard Forest, Petersham, Mass. Salt accumulation and respiration.

Wadleigh, Cecil H., Agent, U. S. Regional Salinity Laboratory, Riverside, Calif. Mineral nutrition.

Wallace, Raymond H., Assoc. Prof. Botany, Univ. of Conn., Storrs, Conn. Physiology; light and radiation.

Walrath, E. K., Agronomist, Eastern States Farmers' Exchange, West Springfield, Mass. Fertilizer; nutrient deficiency diagnosis, and photography; nutrient deficiency symptoms.

Wann, Frank B., Assoc. Prof. Botany, Utah Agri. College; Logan, Utah. Physiology of potato and tomato diseases; chlorosis.

Warner, Robert M., Physiologist, Research Dept., Firestone Plantations Co., Monrovia, Liberia.

Watson, Stanley A., 216 St. James St., Peoria, Ill. Vitamins; protein metabolism; respiration.

Waugh, John G., Kellogg Radiation Laboratory, Calif. Inst. of Technology, Pasadena, Calif.

Weatherwax, Paul, Prof. Dept. Botany, Indiana University, Bloomington, Ind. Growth and anatomical differentiation; photoperiodism.

Weaver, J. E., Prof. Ecology, Univ. of Nebr., Lincoln, Nebr. Plant-soil relations.

Webster, James E., Assoc. Prof. Agri. Chemistry Res., Oklahoma A. and M. College, Stillwater, Okla. Phytochemistry; research on corn, sorghum, soybeans.

Weintraub, Robert L., Smithsonian Inst., Washington, D. C.

Weishaupt, Clara G., Assoc. Prof. Biology, State Teachers College, Jacksonville, Ala.

Welch, Walter B., Asst. Prof. Botany, South. Ill. State Normal University, Carbondale, Ill. Water relations; phytoplanktology.

Welton, F. A., Assoc. in Agronomy, Ohio Agri. Expt. Sta., Wooster, Ohio. Turf grasses.

Went, F. W., Prof. Plant Physiology, Calif. Inst. of Tech., Pasadena, Cal. Physiology of growth and tropisms; hormones.

Werner, H. O., Prof. Hort., Univ. of Nebr., Lincoln, Nebr. Tuberization; heat and drouth endurance; cooking quality of potatoes, and potato, tomato and carrot breeding.

West, Cyril, Ditton Lab., East Malling, Maidstone, Kent, England. Seed germination; plant growth; physiology of senescence.

Wester, Robert E., Bureau of Plant Industry Station, Beltsville, Md.

White, David G., Research Dept., Firestone Plantations Co., Monrovia, Liberia.

White, Philip R., Assoc., Dept. of Animal and Plant Path., Rockefeller Inst. for Medical Res., Princeton, N. J. Plant tissue cultures; tissue physiology.

White-Stevens, Robert H., Res. Instructor, L. I. Veg. Research Farm, Riverhead, N. Y. Plant nutrition and plant biochemistry.

Whitney, John B., California Central Fibre Corp., Pisgah Forest, North Carolina.

Whyte, R. O., Deputy Dir., Imp. Bur. Pastures and Forage Crops, Penglais, Aberystwyth, Wales. Physiology of growth.

Williams, Robert H., University of Miami, Coral Gables, Fla. Physiology of the brown algae; teaching plant physiology.

Williams, W. O., Assoc. in Expt. Sta., Div. of Viticulture, Univ. of Calif., Davis, Calif. Carbohydrate and nitrogen relations.

Wilson, C. C., Instructor in Botany, Biology Dept., Univ. of Georgia, Athens, Ga. Stomatal behavior.

Wilson, Coyt, Instructor in Plant Pathology, Div. of Plant Pathology, University Farm, St. Paul, Minn. Physiology of fungi; growth substances.

Wilson, H. K., Prof. Agronomy, University Farm, St. Paul, Minn. Weed control.

Wilson, J. D., Assoc. Pathologist, Ohio Agri. Expt. Sta., Wooster, Ohio. Environmental factors and plant disease; influence of spray materials on transpiration; evaporation and soil moisture.

Wilson, Lloyd G., 424 Wood St., Muskegon, Mich. Chemical methods in physiological analysis.

Winkler, Albert J., Prof. Viticulture, Univ. of Calif., Davis, Calif. Viticulture; carbohydrate nutrition; climatic influences.

Withrow, Alice P., Research, Dept. of Hort., Purdue Univ., W. Lafayette, Ind. Photoperiodism.

Withrow, Robert B., Research Assoc. Hort., Purdue Univ., W. Lafayette, Ind. Phoperiodism; inorganic nutrition.

Wittwer, Sylvan H., Grad. Res. Asst. in Hort., Univ. of Missouri, Columbia, Mo. Physiology of plant reproduction.

Wolfe, H. S., Head Dept. of Hort., College of Agri., Univ. of Fla., Gainesville, Fla. Tropical horticulture.

Working, Earl B., Research Chemist, S.H.A.D.A., Port-au-Prince, Haiti.

Worley, Clair L., S.H.A.D.A., Bayeux, Cape Haitien, Haiti.

Wright, Kenneth E., Asst. Prof. Botany, R. I. State College, Kingston, R. I. Physiology and ecology.

Wynd, F. Lyle, Asst. Prof. Botany, Univ. of Ill., Urbana, Ill. Plant nutrition and respiration.

Yacknowitz, Samuel B., Walton High School, New York, N. Y.

Yarwood, C. E., Asst. Prof. Plant Path., Univ. Calif., Berkeley, Calif. Physiology of obligate parasites; transpiration.

Yocum, L. Edwin, Assoc. Prof. Botany, George Washington Univ., Washington, D. C. Transpiration and water relations.

Zimmerly, H. H., Director Virginia Truck Expt. Sta., Norfolk, Va. Mineral nutrition; effect of pH on plants.

Zscheile, F. P., Asst. Prof. Agri. Chemistry, Purdue Univ., Lafayette, Ind. Photosynthesis; plant pigments; spectroscopy of biological compounds.

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Quillian, M. C.

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Tiffany, L. Hanford

Jacksonville
Stoldt, Elnore

Lisle
Jurica, Hilary S.

Peoria
MacMasters, Majel M.
Watson, S. A.

Rochelle
Danielson, L. L.

Urbana
DeTurk, E. E.
Dungan, Geo. H.
Hottes, Chas.
Lorenz, Ralph W.
Noggle, G. Ray
Skok, J.
Wynd, F. Lyle

Indiana

Bloomington
Weatherwax, Paul

Indianapolis
Hobbs, C. H.

Lafayette
Carr, R. H.
Girton, Raymond E.
Greene, Laurenz
Hartman, John D.
Hoffer, G. N.
Mullison, W. R.
Withrow, Alice P.
Withrow, Robert B.
Zscheile, F. P.

Notre Dame
Noecker, Norbert L.

Iowa

Ames
Bakke, A. L.
Chang, H. T.
Clark, Norman A.
Gilman, Jos. C.
Loomis, W. E.
McComb, A. L.
Norman, A. G.
Pierre, W. H.
Sprague, G. F.
Sylvain, Pierre G.

Indianola
Doty, H. S.

Iowa City
Craft, J. H.
Loehwing, Walter F.
Marsh, Gordon
Nelson, C. H.

Sioux City
Hoxmeier, Mary Claire

Kansas

Hays
Barton, A. W.

Lawrence
Horr, W. H.

Manhattan
Frazier, J. C.
Miller, Edwin C.
Swingle, Chas. F.

Topeka
Pratt, D. J.

Kentucky

Lexington
Bailey, L. F.
Emmert, E. M.
Fergus, E. N.
Jeffrey, R. N.

Louisville
Bennett, R. E.
Davies, P. A.

Richmond
LaFuze, H. H.

Louisiana

Bogalusa
Sitton, B. G.

New Orleans
Altschul, A. M.
Conrad, C. M.
Pessin, L.

University
Flint, Lewis H.
Kimbrough, W. D.
LeClerg, E. L.
Moreland, C. F.

Maine

Orono
Chandler, Frederick B.
Raleigh, S. M.
Steinbauer, G. P.
Steinmetz, F. H.

Maryland

Baltimore
Macht, David I.

Beltsville
Aldrich, W. W.
Allison, F. E.
Cooley, J. S.
Cullinan, F. P.
Ezell, B. D.
Hutchins, Lee M.
Lauritzen, J. I.
McKinney, H. H.
McMurtrey, J. E., Jr.
Magness, J. R.
Parker, M. W.
Roberts, J. W.
Stuart, N. W.
Thompson, R. C.
Wester, R. E.

Bethesda
Burk, D.
Reid, M. E.

College Park
Appleman, C. O.
Brown, R. G.
DuBuy, H. G.
Fossum, M. T.
Mahoney, C. H.
Schroder, A. L.
Stier, H. L.

Edgewood
Hageman, R. H.

Glenn Dale
Hope, C.
Stoutemeyer, V. T.

Laurel
Hardy, Thora M. P.
Maki, T. E.
Ostrom, C. E.

Riderwood
Livingston, B. E.

Massachusetts

Amherst
Bennett, E.
Bergman, Herbert F.
Clark, Orton L.
DeRose, H. Robt.
Eisenmenger, W. S.
Jones, L. H.
Peters, Chas. A.

Cambridge
Bailey, Irving W.
Castle, Edward S.
Grossenbacher, Karl A.
Thimann, K. V.

Chicopee Falls
Decker, J. P.

Petersham
Gast, P. R.
Vlamis, J.

South Hadley
Eltinge, Ethel T.

Wellesley
Leng, H.
Pulling, H. E.
Skirm, G. W.

West Springfield
Walrath, E. K.

Michigan

Ann Arbor
Gustafson, F. G.

Detroit
McDermott, J. J.

East Lansing
Ball, Chas. D., Jr.
Barr, C. Guinn
Beeskow, H. C.
Dexter, Stephen T.
Harrison, C. M.
Hibbard, R. P.

Muskegon
Wilson, L. G.

Minnesota

Minneapolis
Burr, G. O.
French, C. S.

St. Paul
Brierly, W. G.
Hamm, P. C.
Hart, H.
Harvey, R. B.
Steinbauer, C. E.
Stor, P. W.
Wilson, C.
Wilson, H. K.

Winona
Charles, H.

Mississippi

Columbus
Evans, Clytee R.

Meridian
Lutz, J. M.

State College
Leonard, Oliver A.

Missouri

Columbia
Levitt, J.
Murneek, A. E.
Wittwer, Sylvan H.

Kansas City
Saeger, Albert C.

St. Louis
Glatfelter, Edith E.
Luyet, Basile J.

Montana

Bozeman
Morris, H. E.

Ekalaka
McIlvanie, S. K.

Missoula
Severy, J. W.

Nebraska

Lincoln
Dimond, A. E.
Weaver, J. E.
Werner, H. O.

New Hampshire

Durham
Dunn, Stuart J.
Latimer, L. P.
Phillips, Thos. G.

Hanover
Lyon, Chas. J.

New Jersey

Atlantic City
Banfield, W. M.

Dover
Macy, Paul

New Brunswick
Bear, F. E.
Davidson, O. W.
Reeve, E.
Robbins, W. R.
Shive, J. W.
Small, J. A.
Spencer, E. L.
Sprague, H. B.

Milburn
Hires, C. S.

Princeton
Dawson, R. F.
Shull, G. A.
White, P. R.

Trenton
Hahn, V.

New York

Geneva
Kertesz, Z. I.
Tukey, H. B.

Ithaca
Clark, D. G.
Curtis, O. F.
Hamner, K. C.
Heinicke, A. J.
Lyon, C. B.
Massey, L. M.
Muenscher, W. C.
Platenius, H.
Raleigh, G. J.
Somers, G. F.
Thompson, H. C.

New Springville, S. I.
Foster, A. Alfred

New York City
Burns, Geo. R.
Carey, Cornelia
Cheney, Ralph H.
Clum, Harold H.
Granick, Sam
Kitchen, E. M.
Osterhout, W. J. V.
Trelease, Sam F.
Yacknowitz, Samuel B.

Poughkeepsie
Roberts, Edith

Riverhead
White-Stevens, Robt. H.

Rochester
Goodwin, R. H.

Syracuse
Lodder, Wm. B.
Meier, Henry F. A.

Yonkers
Miller, Laurence P.

North Carolina

Durham
Addoms, Ruth M.
Korstian, C. F.
Kozlowski, T. T.
Kramer, P. J.

Raleigh
Anderson, D. B.
Burkhart, L.
Veerhoff, O.

Greenville
Caughey, M.

Pisgah Forest
Day, D.
Whitney, J. B., Jr.

North Dakota

Fargo
Helgeson, E. A.

Ohio

Cincinnati
Kersten, H.
Sperti, Geo. S.

Cleveland
Bacon, Franklin J.

Columbus
Brown, Howard D.
Burrell, R. C.
Chadwick, L. C.
Childers, N. F.
Meyer, B. S.
Sampson, Homer C.
Scofield, H. T.
Swanson, C. A.
Ting, Sik-Vung

Dayton
Beck, Wm. A.

Doylestown
Mader, E. O.

Hudson
Herrick, Ervin M.

Oxford
Dennison, R.

Wooster
Caroll, J. C.
Gourley, J. H.
Howlett, F. S.
Sayre, J. D.
Thomas, Roy C.
Welton, F. A.
Wilson, J. D.

Zanesville
Post, F. A.

Oklahoma

Norman
Glasstone, V. F. C.
Rohrbaugh, L. M.

Stillwater
Carter, E. P.
Ireland, J. C.
Schultz, Orville C.
Webster, J. E.

Oregon

Corvallis
Atwood, W. M.
Schuster, C. E.

Pennsylvania

Bryn Mawr
Berry, L. J.

Coatesville
Stoller, B. B.

Lancaster
Briggs, G. S.
Street, O. E.

Lewisburg
Eyster, W. H.
Groner, M. G.

Philadelphia
Seifriz, W.
Shirley, H. L.

Pittsburgh
Darbaker, L. K.
Jennings, O. E.

State College
Anthony, R. D.
Huffington, Jesse M.
Mack, W. B.
Popp, H. W.
Sprague, Vance G.
Sullivan, J. T.
Thomas, Walter

Upper Darby
Jones, J. P.

Rhode Island

Kingston
Gilbert, B. E.
Pepkowitz, L.
Schlenker, Frank S.
Smith, John B.
Stuckey, Irene
Wright, Kenneth E.

South Carolina

Charleston
Heinze, P. H.

Clemson College
Armstrong, Geo. M.
Arndt, C. H.
Cooper, H. P.
Musser, A. M.

South Dakota

Vermillion
Eyster, H. Clyde

Tennessee

Jackson
Overcash, J. P.

Knoxville
Meyer, S. L.
Richey, F. D.

Texas

Austin
Goldsmith, G. W.
Lund, E. J.
McAllister, Frederick
Myers, J.
Rosene, Hilda F.

Beaumont
Higinbotham, N.

Brownwood
Smith, C. L.

College Station
Dunlap, A. A.
Eaton, Frank M.
Talley, Paul J.

Dallas
Longnecker, Mayne

Vega
Caird, R. W.

Wichita Falls
Burrows, F. W.

Utah

Logan
Stark, A.
Wann, F. B.

Provo
Harrison, B.
Shaw, S. T.

Salt Lake City
Abersold, J. N.
Hill, G. R.
Owen, F. V.

Vermont

Burlington
Adams, W. R.
Lutman, B. F.
Marvin, Jas. W.

Virginia

Arlington
Culpepper, C. W.

Blacksburg
Hill, H. H.
Shear, G. M.
Vaughan, E. K.

Norfolk
Carolus, Robt. L.
Thornton, S. F.
Zimmerley, H.

Washington

Wenatchee
Gerhardt, Fisk
Harley, C. P.
Lindner, Robt. C.
Overley, Fred L.

Pullman
Biddulph, Orlin
Harvey, Wm. A.
Vandecaveye, S. C.

Puyallup
Daniel, T. W.
Huber, G. A.

Seattle
Rigg, G. B.

West Virginia

Huntington
King, G.

Morgantown
Dustman, Robt. B.
Leonian, Leon H.
Strausbaugh, P. D.

Wisconsin

Madison
Baldwin, Ira L.
Duggar, B. M.
Gerry, Eloise
Graber, Lawrence F.
Roberts, R. H.
Tottingham, W. E.

Wyoming

Cheyenne
Babb, Myron F.

Laramie
Beath, Orville
Northen, Henry T.

Puerto Rico

Cooper, W. C.
Van Overbeek, J.

Territory of Hawaii

Honolulu
Akamine, Ernest K.
Beaumont, J. H.
Clark, Harold E.
Clements, Harry F.
Cooke, D. A.
Engard, Charles J.
Hartt, Constance E.
Linford, M. B.
Mehrlich, F. P.
Moir, W. W. G.
Nightingale, G. T.
Sideris, C. P.

Australia

Brisbane
Francis, Wm. D.

Perth
Teakle, L. J. H.

Belgian Congo

Bambese
Steyaert, Rene L.

Brazil

Rio de Janeiro
Arens, Karl

British West Indies

Jamaica
Meredith, Clifford H.

Trinidad
Mason, Thomas G.

Canada

Kingston
Krotkov, G.

MacDonald College
DeLong, W. A.

Montreal
Cailloux, M.
Gibbs, R. D.
Scarth, G. W.

Vineland Station
Palmer, E. F.
Van Haarlem, J. R.

Ottawa
Davis, M. B.
Marshall, J. B.

Saskatoon
Bibbey, Robt. O.
Patterson, C. F.
Wort, D. J.

Summerland
McLarty, H. R.

Vancouver
Harris, G. Howell

Winnipeg
Buller, A. H. R.
Leach, Wm.
Sackston, W. E.

Ceylon

Talawakelle
Tubbs, F. R.

Cuba

Habana
Beauchamp, C. E.

England

Aberystwyth
Whyte, R. O.

Bangor
Thoday, D.

Cambridge
Blackman, F. F.

Cardiff
McLean, R. C.

Harpenden
Brenchley, Winifred E.
Richards, F. J.
Russell, E. J.

East Malling
West, Cyril

Liverpool
Stirling, Jas.

London
Steward, F. C.

New Castle upon Tyne
Thomas, Meirion

Finland

Helsingfors
Collander, Runar

Haiti

Cape Haitien
Worley, C. L.

Port-au-Prince
Working, E. B.

India

Benares
Singh, B. N.

Ireland

Belfast
Small, Jas.

Dublin
Dixon, Henry H.

Liberia

Monrovia
Tengwall, T. A.
Warner, R. M.
White, D. G.

Mexico

Mexico City
Castaneda, Manuel A.

Peru

Lima
Martin, W. E.

South Africa

Pretoria
Mes, Margaretha

Stellenbosch
Malan, F. P.
Pearse, H. L.

Sweden

Stockholm
Aslander, Alfred

Switzerland

Berne
Schopfer, Wm. H.

Fribourg
Ursprung, Alfred

Zurich
Frey-Wyssling, Alb.

U. S. S. R.

Moskva
Issatchenko, B. L.
Maximov, N. A.
Prianischnikov, D. N.

Venezuela

El Valle
Luis M. de Eleizalde